

UNIVERSITY OF BRISTOL



THE ANNUAL REPORT

OF THE

Agricultural and Horticultural
Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL

1958

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VISCOUNT BLEDISLOE, P.C., G.C.M.G., K.B.E.

In the passing of Viscount Bledisloe in July, 1958, in his 91st year, agriculture and agricultural research and education lost a leading personality, who throughout his long life had laboured to raise the standards of agricultural production in Great Britain and to improve the status and livelihood of the farming community. There can be few important agricultural bodies in this country who have not benefited from his work, either directly or from his activities at a national level. By the general public he will be remembered as a champion of farming in the House of Lords.

Lord Bledisloe was imbued with a deep love of the countryside and of farming, and this was given expression in his activities as landlord, farmer, administrator and educationist. The foundation of his technical knowledge was laid by a diploma course at the Royal Agricultural College, Cirencester, where he gained a silver medal and which, as a Governor, he continued to serve until his death.

Among the many educational bodies on which Lord Bledisloe served may be mentioned the Royal Agricultural Society of England (Vice-President), the Bath and West and Southern Counties Society (President and Vice-President), the Gloucester Root, Fruit and Grain Society (President), Lawes Agricultural Trust, Rothamsted (Chairman), Bristol University Agricultural Committee (Chairman), The British Association for the Advancement of Science, Agricultural Committee (President). He also presided over the Imperial Agricultural Research Conference held in 1927.

The agricultural activities of Lord Bledisloe were not confined to this country. He was a great believer in the development of Commonwealth agriculture : as Governor General of New Zealand he did much to stimulate the industry, and became a figure much loved by New Zealand farmers. During this period he delivered one of the Cawthron Lectures, in which he reviewed the progress of agricultural research in Great Britain. His services to New Zealand agriculture are commemorated by the many foundations, buildings and student awards bearing his name.

Lord Bledisloe's connections with Long Ashton were very close and intimate, coming through both the University, of which he was a Pro-Chancellor from 1935 to 1946, and the Bath & West Society. In the 1920's he was one of the group of landlords and farmers who collaborated in a scheme of the Society for the improvement of poor pastures, which was carried out by the Research Station staff. He also had close personal contacts with the Station in connection with cider and fruit growing. His last official visit to Long Ashton was on the occasion of the Golden Jubilee of the National Fruit and Cider Institute in 1953, when he formally presented the plaque given to the Institute by the Bath & West Society to mark the part played by the Society in the establishment of the Institute.

Lord Bledisloe was the recipient of several University honours. We at Long Ashton are proud to record that our University conferred on him the Honorary Degree of Doctor of Science in 1925 as a fitting tribute to his services to agricultural research and education.

By the death of Lord Bledisloe we have lost an old and honoured friend, who had a lasting interest in the work of the Station and who served for many years as a link with the early pioneers of the National Fruit and Cider Institute.

T. WALLACE.

INTRODUCTION.

By **H. G. H. KEARNS**, Director.

The year proved an exceptionally busy one for the staff owing to the six-yearly inspection of the Station by an A.R.C. Visiting Group in May, the commencement of the building of the new laboratories, offices and refectory, and the serious illnesses of Professor Wallace and Mr. Leggett during the latter part of the year.

The death of Lord Bledisloe, an old and valued friend of the Station, has already been referred to : we are also very sorry to have to record the death of Professor E. J. Maskell, F.R.S., on 20th December, 1958, at the age of 63. Professor Maskell was Mason Professor of Botany, University of Birmingham, and had represented the Agricultural Research Council on the Agricultural Committee of Bristol University since 1956. His kindly interest in the work of the Station, in particular in younger members of staff, was always greatly appreciated. The death of Sir Stephen Tallents in September must also be recorded. His interest in the work of the Station, particularly in the collection of basket and bat willows, had extended over many years.

Professor Wallace continued as Director of the A.R.C. Unit of Plant Nutrition (Micro-nutrients), and also carried out assignments for the Colonial Office and for F.A.O. during the year. At the request of the Colonial Office, he visited the Caribbean area between 21st January and 16th March as chairman of a scientific team appointed to investigate the research needs of the citrus industry in the British Caribbean. The other members of the team were Dr. A. F. Camp, Director Emeritus of the University of Florida Citrus Experiment Station, and Mr. H. R. Hinton, Scientific Adviser's Division (Food), Ministry of Agriculture, Fisheries and Food, London. Jamaica, British Honduras, Trinidad and Florida were visited, and it is rewarding to know that, as a result of the team's report, steps have already been taken by planters in Jamaica to improve the quality and cropping of citrus.

Professor Wallace attended the 6th Congress of the International Potash Institute held in Madrid from 16th to 18th September, where he presented a paper entitled "Potassium uptake in relation to soil moisture." As representative of the A.R.C., he has acted as co-ordinator for an F.A.O. co-operative research project on trace elements. During 1958 he collected and submitted to F.A.O. detailed information from 15 countries from the West European Group and Israel, regarding work in progress on trace elements in relation to the nutrition of crops and livestock. It was proposed to hold a conference in Bristol in 1959 under his chairmanship, in connection with this project, but his severe illness made it necessary to cancel the meeting. Professor Wallace's illness, due to a coronary thrombosis on 25th November, came as a great blow to all his friends, as he had obviously been enjoying a very active semi-retirement and was in the best of health. We were, however, very glad and relieved to know by the end of the year that he was well on the way to complete recovery, and that, after three months' complete rest and a further three months' convalescence, it was likely that he would be able to resume his normal life and continue to fulfil his commitments on various research committees and governing bodies.

We were also very much concerned during November over the unexpected illness of our Secretary, Mr. Leggett. An immediate eye operation became necessary and was carried out on 22nd November. After an anxious period we were very relieved to know that the operation had been successful. His recovery was unfortunately delayed by post-operative complications, but we are very glad that, since his return home at the end of the year, he has made good progress.

These serious illnesses have thrown a heavy strain on both Mrs. Wallace and Mrs. Leggett, who have been much in our thoughts during this time of great anxiety.

The election in July of Mr. R. W. Marsh, Head of the Mycology Section, to a Readership in Plant Pathology in Bristol University gave great pleasure to his many friends. This is only the fourth Readership accorded to a member of the Station staff. Mr. Marsh, who has been a member of the Long Ashton staff since 1926, is well known, both in this country and abroad, as an authority on fungus diseases of fruit crops. He has twice visited Canada as the holder of A.R.C. Travelling Research Fellowships, first for three months and then for a year, and in 1956 he spent three months in Yugoslavia as an F.A.O. visiting specialist to advise on the expansion of the plant protection service. He has been editor of the *Annals of Applied Biology* for many years and a member of the Publication Committee for *Scientific Horticulture*: he has been President of the Association of Applied Biologists and President of the Mycological Society. There are a large number of mycologists working in many parts of the world who owe much of their early training to Mr. Marsh.

The work of the Station and of the A.R.C. Unit of Plant Nutrition (Micro-nutrients) was inspected by an A.R.C. Visiting Group from 28th to 30th May. The Group consisted of the following :—

Mr. David Lowe, C.B.E., Chairman.

Professor T. A. Bennet-Clark, M.A., Ph.D., F.R.S., F.L.S., Department of Botany and Plant Biology, King's College, London.

Professor E. G. Cox, T.D., D.Sc., F.I.P., F.R.I.C., F.R.S., Department of Inorganic and Structural Chemistry, Leeds.

Professor C. W. Wardlaw, Ph.D., D.Sc., M.Sc., Pro-Vice-Chancellor, Department of Botany (Cryptogamic B.), Manchester.

Professor V. B. Wigglesworth, C.B.E., M.D., F.R.S., Department of Zoology, Cambridge.

Dr. S. R. Elsdon, B.Sc., Ph.D., Senior Lecturer in charge of Department of Microbiology, Sheffield.

Mr. W. G. Alexander, Dr. E. E. Cheesman, Mr. G. G. Samuel and Mr. A. Oates of the A.R.C.

A survey of the facilities of the Station and a programme of research for the six year period 1st October, 1958—30th September, 1964, had been prepared by the Director. The Group proposed that the A.R.C. Unit should be disbanded on 30th September, 1959, when Professor Wallace's Honorary Directorship is due to terminate, and, where appropriate, that the staff should be absorbed into the work of the Station. The programme of research work was approved and valuable recommendations were made. The visit was greatly appreciated by the staff.

Two changes in membership of the Agricultural Committee have occurred. Lord Waldegrave has been obliged to relinquish his duties on the committee on his appointment as Parliamentary Secretary to the Ministry of Agriculture, Fisheries and Food, but it is hoped that he will resume them on the completion of his Secretaryship; we should like to congratulate him on his appointment to this high office. We should also like to welcome to the committee the well-known Somerset farmer, Mr. E. M. Owens of Langford, who has been appointed in place of Sir Arthur Hobhouse, who has resigned.

In December 1957, on the recommendation of the Committee of Deans, Senate of the University agreed that holders of permanent appointments at Long Ashton who are given academic status in the University need no longer have the status renewed annually.

The Station's associations with other research centres, the advisory services and the agricultural industry were maintained during the year through conferences, personal visits of staff, and courses conducted at Long Ashton.

The annual Refresher Course for officers of the National Agricultural Advisory Service was held at Long Ashton on 22nd and 23rd July and was attended by 45 horticultural officers of the N.A.A.S. and 16 officers of Local Education Authorities. The co-operation of the N.A.A.S. was enlisted in arranging visits in October by Mr. Cobbald, Mr. Harper, Professor Kearns and Dr. Morgan to a number of fruit farms in Worcestershire and Herefordshire, in connection with methods of low-volume spraying. The N.A.A.S. also assisted Mr. Hobbs in arrangements for a number of the Plantations staff to visit fruit farms and experimental centres in the West Country. Such visits provide an opportunity for scientific and recording staff to keep up-to-date with current developments on fruit farms and to see something of the practical results of research. The Station is grateful to the Governing Committee for approving the cost of these visits and to the fruit growers who welcomed our staff and went to so much trouble to make every visit worth while. It is proposed to arrange these visits each year, and to include members of the scientific staff whose duties do not give them many opportunities of field work.

Plant pathologists and plant protection chemists of the East Malling Research Station visited the Station from 26th to 28th February and held informal meetings with members of the corresponding sections at Long Ashton. We are grateful to the Director of East Malling for this opportunity for a very fruitful interchange of ideas, and hope that it will be possible to arrange for regular meetings.

The annual courses on domestic methods of preserving fruit and vegetables were conducted by Miss Crang and the staff of the Domestic Food Preservation Section between 21st July and 1st August and 25th August and 11th September. The number of applicants who can be accepted for these courses is restricted. Those attending included five county organizers of rural domestic economy, seven lecturers from training colleges, a member of staff from the Ministry's Scientific Adviser's Division, and teachers of domestic science. Four came from Scotland, and others from Thailand, Malaya and South Africa.

Several Long Ashton staff attended the Fifth Easter School in Agricultural Science held at Sutton Bonington School of Agriculture, University of Nottingham, from 14th to 17th April, 1958. Drs. Hewitt and Nicholas contributed papers entitled "Some aspects of mineral nutrition in legumes" and "Some biochemical aspects of nitrogen fixation," respectively.

In view of the great interest that has grown up recently in perry pears in Somerset, facilities have been offered to cider and perry makers for their orchard staff to receive instruction in budding at the Station.

We have been glad to extend to Dr. Battersby, Department of Organic Chemistry in the University, greenhouse facilities for growing plants for experiments with radio-active tracers.

A gift of banana and tea plants was gratefully received from Tilgate Research Station, Sussex, and a consignment of Lacatan banana suckers from the Ministry of Agriculture and Lands, Jamaica. These are being grown in the new greenhouses by the Plant Pathology Section in connection with studies of the effects of petroleum oils on the Leaf Spot disease of bananas. Laboratory facilities in that section were extended, by arrangement with the Banana Board of Jamaica, to Dr. Meredith in connection with investigations on storage rots of bananas shipped to this country.

In the early part of the year the Agricultural Research Council decided that their Technical Committees on Fertilizers and on Research Relating to Fruit Juices and Cider should be dissolved. Professor Wallace had been chairman of the first committee for six years and a member of the second. It was felt that meetings of research workers and others interested in fertilizer problems could be arranged at appropriate times to discuss matters of particular interest, and that problems of the cider industry could be put to research workers through the Cider Committee of the Research Station, on which all the trade members of the A.R.C. Committee are serving. The Station hopes that Professor M. Stacey, F.R.S., University of Birmingham, and Dr. A. H. Cook of the Brewing Research Foundation, Nutfield, Surrey, will pay annual visits to the Research Station, so that the Cider and Fruit Juices Section may continue to profit by their advice and help.

Professor Wallace, on his retirement, resigned from the Joint Editorship of the Journal of Horticultural Science, an office to which he had devoted many years of service. Mr. T. N. Hoblyn of East Malling Research Station remains the sole editor for the time being, and Professor Kearns has accepted an appointment to the Publications Committee.

Professor Kearns has also accepted office during the year as a member of the Pest Infestation Research Board of the Department of Scientific and Industrial Research for the four years commencing 1st April, 1958.

At a staff meeting held on 21st February, 1958, a Laboratory Services Committee was formed under the chairmanship of Dr. J. T. Martin. This committee will receive and make suggestions for improving laboratory services and promoting safe working in the laboratories. It has met several times during the year and shown that it will be of considerable assistance to the Chief Laboratory Steward and the scientific staff.

Staff.

We are very sorry indeed to have to record the death, at his home at Long Ashton on 1st January, 1958, of Mr. F. E. Maltby, Chief Laboratory Steward at the Research Station since 1936. He had been in poor health for a number of years and had carried out his work for some time under severe handicaps. Professor Wallace, under whom Mr. Maltby served for so many years, writes as follows :—

“ Mr. Maltby received his early training in the Botany School, Cambridge University, where he did special work in mycology in addition to general laboratory management. At Cambridge he assisted Professor B. T. P. Barker along with the late Mr. W. Camps, who came to Long Ashton to organize the laboratory services under Professor Barker, and became the first Chief Laboratory Steward at the Station.

Mr. Maltby joined the Long Ashton staff as assistant to Mr. Camps in June, 1914, and succeeded him as Chief Steward on his retirement. As Chief Steward he had heavy responsibilities in the rapidly expanding Station, and he played an important part in the furnishing and equipping of the laboratories in the post-war period. During his early years at Long Ashton he found time to follow his mycological work, in which he was very skilled, and during that period he also developed a process for preserving fruits and foliage in their natural colours.

He always gave of his utmost to the Station, which was his great interest in life. His long and devoted service was officially recognized by the award of the British Empire Medal (B.E.M.) in June, 1955, after he had completed 40 years' service. With Mr. Maltby the interests and welfare of the Station always ranked as the highest priorities in assessing the need for and value of items of equipment. He abhorred any form of waste or misuse of apparatus, maybe a legacy from the early days of his training, when funds for scientific research were meagre. These characteristics were not always appreciated by his colleagues when expensive items of equipment were suggested, but all learned to respect the keen judgment and spirit of service that activated his decisions in such matters. In his passing the Station has lost a loyal servant and his older colleagues a valued friend.

During the First World War Mr. Maltby served abroad in the R.A.M.C., and he had been a keen member of the Long Ashton Branch of the British Legion from its inception. He leaves a wife and one son, who is on the laboratory staff of Messrs. H. W. Carter & Company of Coleford.”

Mr. Maltby has been succeeded as Chief Steward by Mr. George H. Jones, A.R.P.S., who has been a member of the Station staff since 1925. In addition to other duties, Mr. George Jones has developed the photography work of the Station, and was responsible for the colour photographs—over 300 in number—used as illustrations in Professor Wallace's book “The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms.”

We were very sorry indeed to lose Miss M. Sturdy, a much valued member of the staff of the Domestic Food Preservation Section, who resigned on February 28th to marry Mr. John Alvis. After spending the winters of 1942-44 working with the Section, whilst on the staff of the Ministry of Agriculture as Temporary Inspector for their Fruit Preservation Scheme, she accepted an appointment at the Station. She made many contributions to food research, especially in connection with meat preservation and deep-freezing, and she also took a major part in running the annual courses in domestic food preservation conducted by that Section. The Station is also greatly indebted to Miss Sturdy for all her good services in organizing the staff canteen over a number of years; whenever large-scale catering was required for staff functions, Miss Sturdy could always be relied on to give us her invaluable and expert services. We wish her very much happiness in her married life.

We were also sorry to lose Dr. Downing's services on his resignation on

31st August from the Chemistry Section, on his appointment to the Ministry of Supply, Chemical Defence Experimental Establishment, Porton, Wiltshire.

Miss M. Leach, N.D.P., was appointed from 9th June, 1958, to the Domestic Food Preservation Section, following the resignation of Miss Sturdy. Miss Leach comes to us after fourteen years' service as County Instructress in Rural Domestic Economy in Leicestershire and Rutland, and has had wide experience in the preservation of foods.

Appointments to Assistant Staff.

Mr. R. Davenport, Assistant (Scientific), Cider Microbiology, from 14th April, 1958.

Mr. R. H. Davis, Assistant (Scientific), Organic Chemistry, from 17th February, 1958.

Miss S. M. Benson, Assistant (Scientific), Cider Microbiology, from 10th February, 1958.

Miss G. M. Badman, Assistant (Scientific), Cider and Fruit Juices, from 1st June, 1958.

Mr. A. A. Baker, Assistant (Scientific), Entomology, from 27th October, 1958.

Mr. R. D. Child, Assistant (Scientific), Pomology, from 13th October, 1958.

Mr. R. J. Pring, Assistant (Scientific), Analytical Chemistry, from 15th September, 1958.

Miss M. Shellard, Assistant (Scientific), Pomology, from 13th October, 1958.

Miss S. Johnson, Assistant (Scientific), Nutrition of Fruit Plants, from 27th October, 1958.

Miss P. M. Stevens, Assistant (Scientific), Cider and Fruit Juices, from 6th October, 1958.

Miss M. R. Manning, Assistant (Scientific), Physical Chemistry, from 6th October, 1958.

Miss J. Parsons, Assistant (Scientific), Physical Chemistry, from 1st December, 1958.

Return from National Service.

Mr. A. J. Sims, Assistant Experimental Officer, returned to Physical Chemistry on 1st March, 1958.

Resignations from Assistant Staff.

Mr. C. A. Robinson, B.Sc., Assistant Experimental Officer, Greenhouse Officer, Unit of Plant Nutrition, from 31st August, 1958.

Mr. P. E. W. Rodgers, Assistant Experimental Officer, Cider and Fruit Juices, from 31st January, 1958.

Miss K. Guite, B.Sc., Assistant Experimental Officer, Cider Microbiology, from 15th February, 1958.

Mr. A. J. Sims, A.R.I.C., Assistant Experimental Officer, Physical Chemistry, from 31st October, 1958.

Mr. D. J. Jones, Assistant (Scientific), Unit of Plant Nutrition, from 31st July, 1958.

Mr. J. T. Davies, Assistant (Scientific), Physical Chemistry, from 30th September, 1958.

Mr. G. Bradshaw, Assistant (Scientific), Cider and Fruit Juices (not returning to the Station when National Service completed).

Special Training.

Dr. R. J. W. Byrde, Mycology Section, attended a course in biochemical techniques at the School of Biochemistry, University of Cambridge, during November and December. Thanks are due to Professor F. G. Young, F.R.S., and Dr. E. F. Gale for making this possible. The experience gained will assist Dr. Byrde in his work on host/parasite relationships.

Mr. W. J. Redmond, A.R.C. Unit of Plant Nutrition, attended a vacation course in Russian at Birmingham University from 8th to 20th September. His help in translating scientific papers and correspondence in various languages is gratefully acknowledged.

Appointments to Administrative Staff.

Miss V. P. L. White, Machine Operator/Accounting Clerk, from 13th October, 1958.

Miss C. G. Barnett, Shorthand Typist, from 22nd September, 1958.

Miss E. Lawrence, Junior Clerk, from 1st January, 1958.

Resignations from Administrative Staff.

Mrs. J. M. Bendall, Machine Operator/Accounting Clerk, from 30th September, 1958.

Mrs. D. M. Sherborne, Shorthand Typist, from 31st August, 1958.

Visits Abroad.

Dr. D. J. D. Nicholas, by invitation, visited Helsinki and Turku Universities, Finland, from 23rd April to 1st May, to give lectures to students and to the Finnish Agricultural Society.

Dr. L. C. Luckwill, by invitation, attended a Symposium arranged by the Society for the Study of Development and Growth, held at Mount Holyoke College, South Hadley, Massachusetts, U.S.A., from 9th to 11th June.

Miss B. A. Crang attended the 9th International Congress on Home Economics, University of Maryland, near Washington D.C., U.S.A., and the congress tours, from 18th July to 15th August.

Professor T. Wallace, by invitation, attended the 6th Congress of the International Potash Institute, held in Madrid from 16th to 18th September.

Dr. E. J. Hewitt visited the West African Cocoa Research Institute, Ghana, and the West African Institute for Oil Palm Research, Benin City, Nigeria, from 20th October to 21st November, at the request of the Colonial Office.

Mr. H. R. Mapother returned on 23rd March from North Borneo, where, as reported last year, he had been undertaking an investigation into Bunchy Top Disease of *Abaca* (Hemp), at the request of the Colonial Office.

Professor T. Wallace visited the Caribbean Area and Florida, as already reported, from 21st January to 16th March, on behalf of the Colonial Office.

We are grateful to the A.R.C. for travel grants which made possible the following visits by members of staff :—

Dr. C. Bould and Dr. L. C. Luckwill attended the XVth International Horticultural Congress, Nice, France, 11th–18th April.

Dr. W. D. E. Thomas visited Dr. J. Hammelen of Brussels University from 4th to 9th July to discuss his work on the determination of dynamic surface tensions.

Dr. D. Wilson (during his year's Research Fellowship in Canada) attended the Xth International Genetics Congress, McGill University, Montreal, Canada, 20th–28th August.

Dr. D. Woodcock attended, by invitation, the Jubilee Meeting of the American Phytopathological Society, Bloomington, Indiana, U.S.A., from 24th to 28th August.

Dr. D. J. D. Nicholas and Dr. H. E. Davenport, A.R.C. Unit of Plant Nutrition, attended the 4th International Congress of Biochemistry, Vienna, from 1st to 6th September.

Mr. A. J. Abbott, A.R.C. Unit of Plant Nutrition, spent the period 29th September–12th October visiting the laboratories of Professor Gautheret and Dr. Morell in Paris and Versailles, respectively, to study tissue culture methods and problems.

Degrees, Honours, Etc.

Mr. R. W. Marsh was elected to a Readership in Plant Pathology in the University of Bristol, as from 1st October, 1958.

Dr. D. Woodcock was awarded the degree of D.Sc. (Dunelm) for his work on the influence of chemical structure on various types of biological activity.

Dr. D. J. D. Nicholas was awarded the degree of D.Sc. (Lond.) for his work in the field of plant biochemistry.

Mr. N. G. Morgan was awarded the degree of Ph.D. (Bristol) for a thesis entitled "Spray application techniques for crop protection, with special reference to the spray deposits and their biological efficiency."

Mr. D. G. Hill-Cottingham was awarded the degree of Ph.D. (Bristol) for a thesis entitled "A study of some iron chelates used in horticulture."

Mr. J. K. Faulkner, who has been attending a Sandwich Course at the Bristol College of Technology, was awarded the degree of B.Sc. (Lond.) in Honours Chemistry.

Mr. B. L. Davies passed the Graduate Membership examination of the Royal Institute of Chemistry.

Buildings and Land.

The foundations of the new laboratory and office block and the refectory, and the central boiler heating system, were commenced in early September. Almost continuous wet weather held up the work, but it is anticipated that the refectory will be completed by August, 1959, and the remainder of the buildings twelve months later. The new laboratory and offices are sited in the old cider orchard adjoining the main Weston-super-Mare road, and consist of a two-storied building of about 24,000 sq. ft. floor space, which will become the main laboratory of the Research Station.

The refectory is a single-storied building of about 5,000 sq. ft. floor space, sited so that the main dining hall and common room overlook a wide and pleasant expanse of the plantations; about $1\frac{1}{2}$ acres of grass land adjoining the building will add to its amenities. The refectory will not only replace the existing inadequate canteen, but will in addition provide facilities for meetings.

The central oil-fired heating system will provide space heat and hot water for the greenhouse block and associated laboratories, the new laboratories and offices, the refectory, and at a later date, probably the Biology laboratory. This system of central heating will reduce maintenance costs.

The completion of the new buildings will provide much-needed additional facilities for several sections of the Station. The completion of the refectory in 1959 will make it possible to undertake the long-overdue overhaul of the library. During the war the museum-lecture room was converted into a kitchen and small canteen, and later, with increased staff, the library itself was used for meals. It is proposed to redecorate and furnish the library and to convert the canteen to a reading room and library office.

The development of Birdwell Gardens housing estate on land adjacent to the new greenhouse block provided an opportunity for the adjustment of the boundary and made it necessary to erect a long chain-link fence. It will also be necessary to erect an extensive fence around Plot 23 on Fenswood Farm to restrict trespass on experimental plots. The cost of the fencing will be met by a capital grant from the Agricultural Research Council.

A programme of grubbing and replanting of plantations over the next few years has been arranged. It will be possible to segregate many of the entomological and mycological investigations on the north side of the farm, thus avoiding the complications that arise from having a pest-ridden plot adjacent to a pomological one.

Other Activities.

Members' Day.

The Annual General Meeting and Field Day for Members of the National Fruit and Cider Institute was held on 11th July. Exhibits arranged in the Cider House and greenhouses illustrated pollination in cider orchards, mineral deficiencies in fruit plants, basket willow cultivation, and selective weed-killers for nursery beds and black currant plantations. In the afternoon Members toured orchard plots for demonstrations of work on apple mildew.

Cider Sampling Day.

There was a large attendance on Sampling Day, held on 1st May. The morning programme was devoted mainly to single-variety ciders and perries included in the lists of recommended varieties. Other ciders demonstrated some of the effects of biennial bearing, nitrogen dressings and virus infection on cider quality. Demonstrations in the Cider House included exhibits on the detection of *Saccharomyces ludwigii* in bottled ciders, plastic closures for bottles, and the role of chromatography in cider research. The laboratories were open to visitors, and guided tours were arranged in the plantations in the afternoon.

Visitors.

The number of organized parties visiting the Station was 37, compared with 46 in 1957 and 33 in 1956. Visitors included members of the Pathology and Plant Protective Chemistry sections of East Malling Research Station, and Fellows of the Chemical Society, who were holding their 117th Anniversary Meeting in Bristol.

Organized Parties, 1958.

Bath College of Domestic Science.
 Bath Technical College.
 Bedminster Down Secondary School, Bristol.
 Blandford Grammar School, Dorset.
 Bristol Area Produce Guild.
 Bristol Camera Club.
 Bristol Naturalists, Botanical Section.
 Bristol 175th Scout Group, Bushy Park.
 Bristol University, Diploma of Public Health Students.
 Bristol University, Horticultural Science Laboratories Students.
 Bristol University, Honours Botany Students.
 Canon Slade Grammar School, Bolton.
 Chemical Society, Fellows.
 Churchill W.I. Produce Guild.
 Clifton College Natural History Society.
 College of St. Matthias, Barrow Court.
 Colombo Plan Students.
 Domestic Food Preservation Courses (2).
 East Malling Research Station, Pathology and Plant Protection Staff.
 Hereford Training College.
 Institute of Medical Laboratory Technology, Bristol Branch.
 Institute of Medical Laboratory Technology, Conference.
 Institutional Management Association, West of England Branch.
 Irish Horticultural Advisory Officers.
 Ministry of Education, Agriculture and Domestic Economy Panels.
 N.A.A.S. and L.E.A. Horticultural Officers, Refresher Course.
 N.F.U. Worthing and West Sussex Growers.
 Newton Park College, Bath.
 Parnall's Foremen's Association, Fishponds.
 St. Edward's School, Oxford, Farming Club.
 Society of Applied Bacteriology.
 Somerset Farm Institute, Horticultural Students.
 Southmead Estate Community Association, Bristol.
 South Wilts. Grammar School for Girls, Salisbury.
 Sphalerite Society, Avonmouth.
 Veterinary Students' Association.

Visitors from Overseas, 1958.

Australia :

Mr. C. S. Andrew, C.S.I.R.O., Brisbane.
 Dr. D. H. Ashton, Melbourne.
 Mr. N. C. Crowley, Waite Institute, Adelaide.
 Mr. T. A. Frankcomb, Ranelagh, Tasmania.
 Mr. S. E. Hardisty, Perth.
 Dr. L. P. H. Jones, Division of Plant Industry, C.S.I.R.O.
 Mr. R. H. Macdonald, Melbourne.
 Dr. K. D. McLachlan, C.S.I.R.O., Canberra.
 Mr. Bryce C. Rankine, Wine Research Institute, Adelaide.
 Mr. K. N. Shea, Stanthorpe, Queensland.
 Dr. R. J. Swaby, Division of Soils, C.S.I.R.O.
 Mr. G. C. Wade, Hobart, Tasmania.

Argentina :

Mr. P. Seeligmann, University of Tucuman.

British Honduras:

Mr. D. H. Romney, Belize.

Cameroons :

Mr. J. R. Austin, Buea.

Mr. S. J. Evans, Plant Protection Ltd.

- Canada :* Mr. F. E. Atkinson, Summerland, British Columbia.
 Dr. H. A. Daubeney, Agassiz, British Columbia.
 Dr. H. Hill, Division of Horticulture, Ottawa.
 Mr. W. Kalbfleisch, Ottawa.
 Miss C. MacFarlane, Halifax, Nova Scotia.
 Mr. A. D. McMechan, Department of Agriculture.
 Mr. C. C. Strachan, Morden, Manitoba.
 Dr. and Mrs. W. H. Upshall, Vineland, Ontario.
- Ceylon :* Mr. L. B. Chandrasekera, Rubber Research Institute, Agalawatta.
 Mr. L. H. Fernando, Department of Agriculture.
 Mr. D. G. Nethsinghe, Coconut Research Institute.
 Dr. T. Visser, Tea Research Institute.
- Chile :* Mr. D. Pavez de Saa, Ministry of Agriculture.
- Denmark :* Mr. H. Stimming, Santiago.
 Mr. J. V. Christensen, Blangstedgaard.
 Dr. N. Dullum, Blangstedgaard.
 Mr. E. Kirt, Copenhagen.
 Mr. E. Nøddegaard, Lyngby.
- Eire :* Dr. E. J. Clarke, University College, Dublin.
 Mr. A. Turner, Department of Agriculture, Dublin.
- Finland :* Miss K. Salokangas, Piikkio.
- France :* M. R. Bernhard, Pont de la Maye.
 Dr. G. Carpeni, University of Marseilles.
 M. M. Contanceau, Versailles.
 M. A. Reifenberg, Jagny sous Bois.
 M. P. R. Remy, Pont de la Maye.
- French Cameroons :* M. J. Cuillé, I.F.A.C.
- French W. Indies :* M. H. Guyot, I.F.A.C.
- Germany :* Professor G. Büchloh, University of Bonn.
 Dr. G. Glander, N.D.K., Hanover.
 Dr. G. Grüner, Hanover.
 Professor F. Hilkenbäumer, University of Bonn.
 Dr. E. E. Kessler, Marburg/Lahn.
- Ghana :* Mr. R. R. Cunningham, W.A.C.R.I.
 Mr. D. A. Wheatley, Kumasi.
- Greece :* Mr. E. Mastrandreu, Ministry of Agriculture.
- Holland :* Mr. J. van Beek, Putten.
 Mr. A. Kleinendorst, Wageningen.
 Dr. D. A. van Schreven, Kampen.
- India :* Dr. G. M. Das, Tocklai, Assam.
 Dr. S. K. Dutta, Tocklai, Assam.
 Dr. J. Kanwar, Punjab.
 Mr. L. B. Singh, Horticulture Research Institute, Saharanpur, U.P.
 Dr. S. M. Sircar, University of Calcutta.
- Iraq :* Dr. H. A. C. McKay, Baghdad.
- Israel :* Mr. H. Brisker, Ministry of Agriculture.
 Mr. Gideon Cohen, Ministry of Agriculture, Jaffa.
- Jamaica :* Mr. R. F. Innes, Mandeville.
 Mr. R. L. M. Kirkwood, Kingston.
 Mr. R. Leach, Kingston.
- Japan :* Dr. M. Fukaya, Tokyo.
 Dr. S. Funahashi, Tokyo University.
- Kenya :* Dr. E. D. Bumpus, Kitale.
 Mr. G. T. Chamberlain, E.A.F.F.R.O., Kikuyu.
 Mr. H. R. Evans, Department of Agriculture.
 Dr. J. Nutman, Nairobi.
 Mr. R. Strange, Kitale.
 Mr. T. Mytton Watson, Nakuru.
- Malaya :* Mr. R. Irving Bell, Klang, Malacca.
 Mr. J. Bolton, Rubber Research Institute.
 Mr. Philip Lai, Malacca.
 Mr. G. Owen, Rubber Research Institute.
 Mr. M. Rashdan, Department of Agriculture.
- Mauritius :* Mr. D. H. Parrish, Sugar Research Institute.
- New Zealand :* Dr. and Mrs. M. M. Burns, Lincoln College.
 Mr. R. M. Davison, Auckland.
 Mr. C. V. Fife, Massey Agricultural College.

	Mr. and Mrs. P. Tait, Nelson.
	Mr. A. D. Thomson, D.S.I.R., Lincoln.
<i>Nigeria :</i>	Mr. and Mrs. F. A. Adeyemi, Ibadan.
	Mr. A. Jefferiss, Kaduna, Northern Nigeria.
	Mr. C. W. Michie, Kaduna, Northern Nigeria.
	Alhaji the Hon. Mohammed Mustafa, Kaduna, Northern Nigeria.
	Mr. C. H. Obihara, Umuahia, Eastern Nigeria.
	Dr. J. Robinson, Ibadan, Western Nigeria.
<i>Pakistan :</i>	Mr. A. R. Azmi, University of Dacca, East Pakistan.
<i>Rhodesia :</i>	Professor E. H. Harper, Salisbury.
	Mr. H. H. Roberts, Department of Agriculture, Northern Rhodesia.
<i>Sierra Leone :</i>	Mr. J. W. O. Jeffery, Rokupr.
<i>South Africa :</i>	Mr. S. C. Cadman, Knysna, C.P.
	Mr. P. M. Grant, Noordrand.
	Mr. J. H. Grobler, Potchefstroom.
	Professor J. E. Kench, University of Cape Town.
	Mr. B. H. Koeppen, Stellenbosch.
	Dr. A. G. Marais, Stellenbosch.
	Mr. E. S. Redelinghuys, Cape Town.
	Mr. H. Shaw, Wattle Research Institute, Pietermaritzburg.
	Mr. D. M. Tanner, Elgin, C.P.
<i>Spain :</i>	Dr. Antonia Medina, Madrid.
<i>Sudan :</i>	Dr. S. O. S. Dark, Shambat.
	Dr. A. D. Hanna, Gezira Research Station, Wad Medani.
<i>Sweden :</i>	Dr. N. Katunuma, Nobel Institute, Stockholm.
<i>Switzerland :</i>	M. and Mme. A. Dufour, Geneva.
	Dr. Hans Lüthi, Wädenswil.
<i>Tanganyika :</i>	Mr. J. M. Hunter, Oldeani.
<i>Trinidad :</i>	Dr. G. K. Maliphant, I.C.T.A.
	Mr. R. Nicholls, I.C.T.A.
<i>Uganda :</i>	Mr. and Mrs. G. V. Coles, Kampala.
<i>U.S.A. :</i>	Mr. R. K. Eskew, U.S. Department of Agriculture, Philadelphia.
	Mr. and Mrs. R. M. Gilmer, Geneva, New York.
	Dr. A. H. Gold, University of California, Berkeley.
	Miss Natalie Gundry, Cornell University.
	Dr. and Mrs. L. G. Holm, University of Wisconsin.
	Mr. R. Hopp, University of Vermont, Burlington.
	Professor O. Lilleland, University of California, Davis.
	Mr. F. S. O'Rourke, East Lansing, Michigan.
	Dr. W. Siegelman, Beltsville.
	Dr. F. L. Stark, Stamford, Connecticut.
	Dr. C. E. Yarwood, University of California, Berkeley.
<i>U.S.S.R. :</i>	Mr. B. P. Pleshkov, Agricultural Academy, Moscow.
<i>Yugoslavia :</i>	Mrs. Blanka Arcanin, Zagreb.
	Mrs. Danica Gajic, Belgrade.
	Dr. M. Perisic, University of Belgrade.
	Miss A. Stojkowska, Skopje.
<i>Zanzibar :</i>	Mr. J. Jago, Government Chemist.

Shows and Demonstrations.

Exhibits were staged during the year at the following shows :—

Royal Horticultural Society, Chelsea, May 20th—23rd.

Royal Agricultural Show, Bristol, July 1st—4th.

Students and Voluntary Workers.

Dr. Milivoje Perisic, Senior Lecturer in Phytopathology, Faculty of Agriculture, University of Belgrade, worked with the Plant Pathology Section from 29th January to 28th May.

Mr. R. Spencer spent a period of training in micro-nutrient techniques under Dr. Hewitt's supervision from May to November, at the request of the Colonial Office, preparatory to taking up an appointment as Plant Physiologist in the Federation of Nigeria.

Dr. D. S. Meredith was seconded from the Research Department of the Banana Board of Jamaica from August, 1958, to March, 1959, in connection with investigations of the banana fruit rots that develop during transit.

Mr. G. T. Brice, a nominee of Somerset County Council, held the Memorial Studentship in Cider-making for the six months commencing 1st October, 1958.

Mr. C. A. Fewson and Mr. G. C. Walker, graduates of Nottingham University and holders of two-year scholarships from the Ministry of Agriculture, Fisheries and Food, began in October the first year of their work under the direction of Dr. Nicholas, on the biochemistry of micro-organisms.

Mr. Shaheen Pasha from Syria, holder of an F.A.O. Fellowship in Horticulture, spent a week in September working in the nursery, by arrangement with the Ministry of Agriculture.

Dr. R. O. Sharples, the holder of the Boots' Research Scholarship, completed his investigations under the supervision of Mr. Marsh on 30th June, 1958. He was awarded the degree of Ph.D., Bristol University, for a thesis entitled "Gloeosporium disease of apple."

Dr. R. T. Burchill, a student from the Horticultural Science Laboratories of the University, completed his studies under the direction of Dr. Bond and Mr. Marsh on 26th September, 1958. He was awarded the degree of Ph.D (Bristol) for a thesis entitled "Studies on apple mildew."

Mr. B. P. Tamayo of the Philippines and Mr. C. V. Govindaswamy of Madras, India, also students from the Horticultural Science Laboratories, Bracken Hill, Bristol, spent part of their course in the Plant Pathology Section.

Mr. E. D. Evens, B.Sc. (Bristol), on his retirement from the College of Technology, volunteered to assist Dr. Woodcock, and the Station gratefully acknowledges his work in synthetic organic chemistry.

In addition to the above, the following postgraduate students continued their investigations at the Station during the year :—

Miss M. F. Roberts, holder of the Boots' Scholarship 1957/59 : Chemistry Section.

Mr. C. F. Cresswell, Rhodes University, South Africa : A.R.C. Unit of Plant Nutrition (Micro-nutrients).

Mr. Khan Afridi, Aligarh Muslim University, India : A.R.C. Unit of Plant Nutrition (Micro-nutrients).

Mr. M. W. Case, holder of a Ministry of Agriculture scholarship : Pomology Section.

Mrs. V. Thisyamondala of Thailand, Colombo Plan trainee, who received part of her training in 1957, completed her course with the Domestic Food Preservation Section in June, 1958.

Social Activities.

The Recreational Club, under the Chairmanship of Dr. Bennett, arranged a variety of social activities, including talks on natural history and travel, visits to theatres and places of local interest, lunch-time gramophone recitals, etc. The Club also organized the Christmas Party, held again at Messrs. Wills' Recreation Hall, Bedminster, on Tuesday, 23rd December. Membership of the Club has risen during the year to 193 and, with the facilities that will be

available when the new refectory is completed, the Club looks forward to increasing its activities. Financial assistance generously provided by the Finance and Business Sub-Committee is gratefully acknowledged.

Membership of the Tennis Club was higher than ever before and, with two hard courts in use, more tennis was played than in previous years. The Men's Team maintained its position in the Bristol and District League, again finishing in third place in Group V. This year the Ladies' Team played a full list of league fixtures. Mr. Stott, representing the club in the League Men's Singles, reached the Quarter Finals. Fifteen friendly matches were played, with wins in five.

The Cricket Club had an unusually strong team and won its way to the semi-final of the Bristol Evening Post "H.E.R." Cup Competition. Of the eighteen matches played during the season, ten were won, seven lost and one drawn.

The Rifle Club continued to use the O.T.C. indoor range at the University during the winter months, and a number of matches and competitions were arranged. The outdoor range at the Station was brought into use during the summer for small-bore rifle shooting, and full-bore shooting was enjoyed at the Pilning ranges. The Station range has now been approved for full-bore pistols at 20 yards. The N.R.A. Donegall Badge for 1957 was won by Mr. C. P. Lloyd-Jones and for 1958 by Dr. A. T. K. Corke.

The Table Tennis Club has doubled its membership in the past year, with a total of forty-two players. Owing to the increased membership another table was purchased early in 1959. A friendly match against the N.A.A.S. at Westbury was much enjoyed.

The Welfare Committee, under the chairmanship of Dr. Bennett, revised its terms of reference during the year, which included the handing over to the Recreational Club responsibility for social activities, but assuming responsibility for the use and amenities of the Commonroom in the Refectory.

The Canteen Committee, under the chairmanship of Dr. Woodcock, in addition to its normal function of organizing the catering for staff lunches throughout the year, once again arranged a very successful Christmas Dinner for about 150 members of staff on Tuesday, 23rd December.

The annual sports fixture with the National Vegetable Research Station, Wellesbourne, took place at Wellesbourne on 28th June, and was followed in the evening by a visit to the Memorial Theatre, Stratford-on-Avon. Thanks are due to Dr. Philp and his staff for providing a most enjoyable day.

Acknowledgments.

The Station is indebted to many members of staff who give so much of their time for the benefit of their colleagues. These include Dr. Bennett, Chairman of the Recreational Club and Welfare Committee, Dr. Byrde, Chairman of the Library Committee, and Dr. Woodcock, Chairman of the Canteen Committee. Special thanks are due to Miss Karen Taylor and Miss Anne Turner, who once again arranged the meals and ordered provisions for the canteen. To Mrs. King and Mrs. Duddridge must be accorded the grateful thanks of the staff for the excellent cooking of the canteen meals.

Mr. Greenwood undertook once again the onerous task of editing the Annual Report in addition to his numerous other duties.

During the year a very heavy programme was undertaken by the Workshops on maintenance and special work. Thanks are in particular due to Mr. S. E. Cole, Mr. H. J. Jones and their staffs for the satisfactory completion of the work. Many members of staff are again indebted to Dr. Morgan for his advice and assistance

Thanks are due to Miss Simpson and Mr. Leggett for their assistance in preparing for the visit of the A.R.C. Visiting Group.

SUMMARY OF RESEARCH, 1958.

Pomology and Plant Breeding.

Plant Breeding.

During the absence of Dr. Wilson in Canada work in plant breeding was confined to routine recording and maintenance of the breeding collections.

Growth Substances.

Using ^{14}C -labelled compounds, a study of the metabolism of 2:4-dichlorophenoxyacetic acid by closely related resistant and susceptible varieties of fruit plants has been carried out in co-operation with the Chemistry Section. Some work with carboxyl-labelled 1-naphthalene acetic acid (NAA) has also been done.

Previous experiments on the mechanism of apple thinning by NAA had suggested that the seed abortion which follows NAA application might be associated with a temporary restriction of carbohydrate supply to the fruitlets. To determine whether NAA itself reduces carbon fixation, treated and untreated apple leaves were exposed in light to an atmosphere enriched with $^{14}\text{CO}_2$: no differences were found.

To localize the effect of NAA and 1-naphthalene acetamide (NAm) in fruit thinning sprays to the vicinity of the fruiting spurs and thus avoid damage to the vegetative growing points, sprays were applied to Laxton's Superb, Crawley Beauty and Cox's Orange Pippin apples at early pink cluster, when spur leaves had unfolded but vegetative buds were only just breaking. Satisfactory thinning was achieved with 100 p.p.m. NAA to 200 p.p.m. NAm, but without significant increase in fruit size.

If only the upper or lower halves of Merton Worcester apple trees were sprayed with 50 p.p.m. NAA soon after petal-fall, it was found that, although the thinning effect was confined to the sprayed half, the size of the fruits on the unsprayed portion was increased.

After hand thinning, varieties showed considerable differences in fruit growth curves, the time and extent of fruit drop, and in embryo weight at maturity.

Gibberellic acid was found to induce parthenocarpy when sprayed at 100 p.p.m. on pot trees of Miller's Seedling at full bloom; pollination was prevented by excision of the styles before the flowers opened. The fruits were more elongated than normal, but grew at the same rate and matured at the same time as normal fruits. Mixtures of various synthetic auxins, either alone or in combination with gibberellic acid, did not induce parthenocarpy. Application of the same treatments to the pear varieties Glou Morceau and Comice was unsuccessful.

Sprays of gibberellic acid did not affect the growth of maiden apple trees. On black currants the compound stimulated the development of the axillary buds on the new growth.

In co-operation with the Department of Pomology at Davis, California, a study was made of the endogenous auxins in two varieties of fig in relation to seed and fruit development. The results showed that, as in the apple, auxin production in the seed was closely associated with endosperm development. Auxin concentration during early fruit development was very low,

rising to a sudden peak at the time of cytokinesis in the endosperm and thereafter declining as the primary endosperm was digested by the growing embryo. A similar auxin peak in the naturally parthenocarpic Mission fig was found to be associated with the development of a parthenogenetic endosperm. There was no correlation between the growth rate of the fruit and its auxin content and it appeared that the chief function of this seed auxin was the prevention of fruit abscission.

To investigate the sensitivity of plants to auxins applied in different environmental conditions, batches of *Coleus* plants were grown under four different combinations of day length (8 or 16 hours) and light intensity (1,500 or 500 ft. candles from fluorescent tubes); a day temperature of 25°C was maintained for 8 hours in each chamber and a night temperature of 16°C for 16 hours. Sensitivity of the plants to auxin was measured by applying known quantities of 2:4:5-trichlorophenoxyacetic acid to excised nodes maintained in a dark room at 25°C and noting the time to abscission of the petioles. Sensitivity to auxin was found to be favoured by low light intensity and short days, approximately four times as much auxin being required to produce the same response on the long day/high light plants as on the short day/low light plants.

Field trials on the control of *Convolvulus arvensis* in black currants with γ -(2:4:5-trichlorophenoxy)-butyric acid (2:4:5-TB) and the related γ -(4-chloro-2-methylphenoxy)-butyric acid (MCPB), showed that the latter compound was too damaging to the currants to be used before picking. With 2:4:5-TB good control of bindweed was obtained, without damage to the bushes, on plots sprayed both before and after picking. Both compounds were applied at 1,000 p.p.m. Regeneration of bindweed from heavily infested plots sprayed in 1957 with 2:4:5-TB at 500 to 1,500 p.p.m. was negligible.

In comparisons of the effects of 2:2'-dichloropropionic acid (dalapon) and 2-chloro-4:6-bis-(ethylamino)-triazole (simazin) on couch grass amongst cordon apples, it was shown that simazin at 5 lb./acre was not effective, but dalapon at 5 lb., and particularly at 10 lb./acre, was very effective in checking the couch grass without damage to the trees.

Physiology of Fruit Plants.

In co-operation with the Chemistry Section a special chamber was developed in which individual attached leaves of apple could be exposed to small quantities of $^{14}\text{CO}_2$, resulting in the formation of labelled photosynthates whose subsequent movement in the tree could be followed by autoradiographic techniques.

An experiment is in progress to investigate the interactions of light and temperature on the vegetative growth of the apple, using the controlled environment chambers completed during the past year.

Variety Trials and Cultural Experiments.

The results so far obtained in the experiment laid down in 1947/48 to compare the behaviour of Cox's Orange Pippin and Winston planted (i) as bush trees at 24 ft. apart; (ii) as bush trees at 24 ft. interplanted with fillers at 12 ft. apart; (iii) as dwarf pyramids at 8 ft. $\times$ 4 ft. and (iv) as cordons at 8 ft. $\times$ 3 ft. were published during the year (26)*. All fillers except those in the centres of the squares of bush trees were removed after the 1957 harvest.

* See Publications, 1958, pp. 186-188.

Because of building operations, observations on the effect of chemicals on the breakdown of apple tree stumps and roots, left *in situ* after tree felling, had to be discontinued in September, 1958. Twenty-four trees had been treated in 1954 with either aqua regia, caustic soda (flake), bleaching powder or a 3:1 Nitrochalk-superphosphate mixture, introduced into holes drilled in the stumps. No treatment had caused a complete breakdown of the stumps; the greatest effect was produced by the fertilizer mixture, which caused a marked softening of the wood (p. 59).

Rootstocks and Propagation.

Further investigations on the "rosette" disease of maiden pears worked on Quince stocks, described in the Annual Report for 1956, have failed to confirm the suspected virus nature of the disease, all attempts to transmit the rosette condition from diseased to healthy material by bud inoculation having failed. It has also been established that no association exists between the rosette and the ringspot virus, prevalent on perry pears. Transplantation experiments in which rootstocks and bud-wood from the same sources were planted and worked in a number of different nurseries have shown that some nurseries consistently produce a high and others a low proportion of rosetted trees, irrespective of the source of the propagating material.

The use of *Malus platycarpa* as an indicator for apple viruses has revealed the presence of a latent ring-spot virus in many commercial apple varieties and rootstocks. Some of the latest rootstock selections are being tested in collaboration with East Malling Research Station.

Investigation of the pendulous growth of a Bristol Cross pear headworked on Laxton's Superb in a nursery near Nottingham, has proved it to be graft-transmissible and therefore presumably due to virus infection. The virus is distinct from that causing rubbery wood in apples. Apple rubbery wood virus has, however, been found in the perry pear Aylton Red, a variety with a naturally drooping habit of growth.

Trees of Cox's Orange Pippin, Worcester Pearmain and Lord Lambourne on two apomictic seedling rootstocks (*Malus toringoides* and *M. sikkimensis*) and M.II produced their second crop during the past season. Cox and Lambourne cropped more heavily on the apomictic stocks than on M.II, whereas the reverse was true for Worcester Pearmain, as in the first cropping season. The mean crop weights in lb. per tree for Cox in 1958 were 8.9 on *M. toringoides*, 8.2 on *M. sikkimensis* and 7.7 on M.II. *M. toringoides* appears to be of interest as a stock which combines precocity with great vegetative vigour.

Cider and Perry Varieties.

Renewed interest in the commercial production of perry has given increased emphasis to problems concerned with the propagation and orcharding of perry pears.

The survey of farms growing perry pears was continued, 107 farms in South Gloucestershire being visited during the year (p. 67). Several varieties of merit, previously unrecorded, were found. Further pomological descriptions of perry pears were completed for publication (p. 70).

A scheme for the distribution of perry propagating material to Station members was initiated, and 2,400 bud stocks of named varieties were provided

during the summer. In a perry pear rootstock and stembuilder trial the variety Pine produced the most satisfactory seedlings for use as rootstocks.

The survey of headworked trees in cider orchards in Herefordshire and Somerset was continued and the widespread occurrence of virus symptoms noted in 1957 was confirmed. The routine virus indexing of cider varieties at Long Ashton was continued and a start was made with the distribution to growers of virus-tested scion material of certain of the varieties on the recommended list (p. 64).

Although 1958 was a year of profuse blossom, the flowers of many cider varieties exhibited abnormalities, such as malformation of stigmas and anthers, which interfered with pollination studies on cider apples. In an experiment carried out in collaboration with the Plant Nutrition Section the effects of various mineral deficiencies on the time of flowering and on pollen viability of Cox's Orange Pippin and Worcester Pearmain were investigated. Considerable differences were seen in the time of flowering, the order of flowering being -Mg, -K, -N, with -P and controls flowering last. No significant differences in pollen germination or tube growth were found.

Studies on fruit set and seed content of the variety Michelin at different distances from a pollen source were continued. As in previous years the maximum effective distance of the pollen source was found to be about 90 feet. (p. 61).

Plant Nutrition : Fruit.

Field Experiments.

The long-term manurial and cover crop experiments at Long Ashton and the N.A.A.S. Experimental Horticulture Stations at Efford and Luddington have been continued. At Efford the first phase of the strawberry factorial manurial experiment has been completed (p. 82). In the black currant manurial experiment significant yield responses were obtained from phosphatic and potassic fertilizers but not from ammonium sulphate. Annual dressings of ammonium sulphate, at the rate of 9 cwt. per acre since 1954, had reduced the soil pH from 6.5 to 5.5 : this reduction in soil pH is probably responsible for the lack of response to nitrogenous fertilizer. Applications of dolomitic limestone have been made to raise the pH to its original value.

No significant response has yet been obtained to fertilizer treatments in the $N \times P \times K$ factorial manurial experiment on black currant at Luddington.

A new pear cover-crop-manurial experiment has been started at Efford in collaboration with East Malling Research Station.

Cover crops continued to have a marked effect on tree growth, leaf nutrient status and yield of fruit of the young apples in the trial at Long Ashton (Plot 18). Trees in a clover (plus grass) cover were more vigorous, had a higher nitrogen status and gave greater yields of fruit than trees under continuous arable culture. Timothy grass and perennial rye grass continued to have a deleterious effect on tree growth, leaf nitrogen and yield of fruit ; trees on these plots responded to additional supplies of nitrogenous fertilizer.

In collaboration with the N.A.A.S. (Reading) a new field experiment was started at Harwell to test the effect of varying amounts of Chel-138 Fe (ethylenediamine bis (α -hydroxyphenylacetic acid)) as a soil dressing and, together with Greenz-26 (an iron complex of ammonium lignin sulphonate) and Fe-HEEDTA (iron chelate of N-hydroxyethyl-ethylenediamine triacetic

acid), as a foliar spray for the control of lime-induced chlorosis. The treatments were applied too late in the season to have any marked effect on chlorosis but leaf analyses indicated that treatments had considerably increased the iron concentration in leaf dry matter.

Pot Experiments.

Further sand culture experiments were made to determine the phosphorus requirements of strawberry, var. Royal Sovereign, using leaf analysis as an index of nutrient status. From a factorial combination of high and low nitrogen with five levels of phosphorus in the nutrient solution, it was found that growth and yield of fruit were severely restricted when the phosphorus concentration at the early fruiting stage was less than 0.2% in leaf dry matter. As the concentration increased from 0.2 to 0.3% P there was an increase in the yield of total fruit and the dry weight of tops, but no significant change in the yield of Grade I fruit. Increasing the concentration beyond 0.35% P caused no further increase in the yield of fruit or total dry matter. These results suggest that the optimum phosphorus concentration at the early fruit ripening stage is about 0.3% P in leaf dry matter. This confirms previous work with the variety Talisman.

A further series of pot cultures with Talisman was vitiated by insufficient winter chilling of the plants, which appear to require a longer period of cold than Royal Sovereign. Early growth was stunted, petioles were short and laminae were small; normal growth was resumed only at fruit ripening.

Preliminary trials were made to propagate apples from leaf-bud and shoot-tip cuttings for trace element studies, and for work on root cation exchange capacities in relation to nutrient uptake.

Laboratory Investigations.

Work on leaf analysis as a guide to the nutritional status of fruit crops was continued. Errors of sampling and chemical analysis were determined for apple, black currant and strawberry. A variance-component analysis was carried out on the major nutrient elements in each crop. It was found that the overall sampling error (based on ten replicate field samples), expressed as the coefficient of variation, was of the order of 6%, of which about 1.2% occurred at the analytical stage, 1.0% at the sub-sampling stage and 3.8% at the field sampling stage.

Further work was done on methods for determining the cation exchange capacity of roots. Exchange capacities have been measured on dried ground roots, using a column percolation technique involving saturation with hydrogen ions followed by potentiometric titration, and on living roots, by saturating first with the appropriate cation, then with hydrogen ion, and measuring the amount of cation exchanged. Attempts to correlate root exchange capacities with nutrient uptake by maiden apple rootstocks have so far yielded negative results, but the picture is complicated by the large reserves of nutrients in the rootstocks. Results with seedling lupin and wheat plants have been more promising.

Analyses of xylem sap from terminal shoots of trees in the apple cover-crop experiment indicated that the nitrogen content of the sap prior to blossoming was positively related to the general nitrogen status of the tree as determined by leaf analysis in the previous season.

Work on the behaviour of iron chelates in calcareous soils was brought to a conclusion with experiments using the chelating agents known by the code numbers RA 156, RA 157 and RA 159, all of which contain phenolic groupings. In collaboration with the Chemistry Section, solutions of these three iron chelates labelled with ^{59}Fe were added to samples of a highly calcareous Evesham clay. Aqueous extracts of the treated soil showed that the behaviour of these iron chelates was similar to that previously found for the related compound Chel-138 Fe, in that no replacement of chelated iron by soil calcium took place. However, some sorption by the clay fraction occurred, and after 30 days the recoveries of Fe-RA 156, 159 and 157 were approximately 50%, 60% and 85% respectively. Little or no isotopic exchange took place with these iron chelates.

Preliminary investigations were carried out on the behaviour of iron chelates within plants, with particular emphasis on the fate of the amino-carboxylic acid residue after removal of the chelated iron. This work has been greatly assisted by the use of ^{14}C -labelled ethylenediamine tetraacetic acid and cyclohexane *trans* 1:2-diamino tetraacetic acid. In collaboration with the Chemistry Section attempts have been made to extract and identify the chelates or their metabolites from plant material by means of ion exchange resins, electrodialysis and paper chromatography.

Chemistry.

Organic Chemistry.

Investigations of the relationship between molecular structure and parthenocarpic activity were continued. 3-substituted 2-naphthyloxyacetic acids were synthesized and tested, but no correlation was found between the electronegativity of the substituent group and biological activity. During attempts to obtain 3-iodo-2-naphthol in improved yield, an example of hydrogen-lithium exchange taking place in preference to halogen-lithium exchange was observed when treatment of 3-bromo-2-naphthol with *n*-propyl-lithium followed by iodine gave only 3-bromo-1-iodo-2-naphthol. The preparation of ten new 3-substituted 2-naphthyloxyacetic acids has been described (46).

In further investigation of the roles played by chelation and lipoid solubility in the fungistatic activity of 8-hydroxyquinoline, routes to the preparation of a series of 6-alkyl-8-hydroxyquinolines were explored.

In work on the metabolism of organic compounds by micro-organisms the formation of γ -(6-hydroxy-2-naphthyloxy)- β -hydroxy-*n*-butyric acid as an intermediate in the β -oxidation of γ -(6-hydroxy-2-naphthyloxy)-*n*-butyric acid by *Aspergillus niger* was established with reasonable certainty by a comparison of ultraviolet absorption spectra of the isolated metabolite and the synthetic acid. Owing to the labile nature of this compound neither material was obtained analytically pure, but work is continuing. Although there was little evidence of ring fission in the metabolism of ω -(2-naphthyloxy)-*n*-alkyl-carboxylic acids by *A. niger* and *Sclerotinia laxa* this process is essential for the formation of 4-methoxysalicylic acid, the only metabolic product of 2-methoxynaphthalene which has been isolated from *A. niger*. The metabolism of potential precursors of 4-methoxysalicylic acid is being investigated and indications of the presence of 7-methoxycoumarin on chromatograms support the postulated

pathway via 2-hydroxy-4-methoxy-*cis*-cinnamic acid. The formation of 4-ethoxysalicylic acid by *A. niger* from 2-ethoxynaphthalene has been confirmed and this conversion is of interest because the metabolite possesses greater fungistatic activity than the substrate (p. 110).

Work on the metabolism of 3-indolylacetic acid by *A. niger* was begun ; there are indications that hydroxylation takes place in both 5- and 7- positions, accompanied by extensive ring fission.

Structural investigations were made of an unknown compound obtained from *Lonchocarpus* resin by a column chromatographic technique.

Physical Chemistry.

Radio-active tracer work with ^{14}C has been extended considerably during the year. In collaboration with the Pomology Section a study was made of the metabolism of ^{14}C -labelled 2:4-D by the leaves of apple, currant and strawberry varieties in relation to their resistance to the herbicide. When treated with carboxyl-labelled 2:4-D, resistant varieties induced rapid decarboxylation, approximately 50% of the carboxyl carbon being evolved as CO_2 in a few days. During the same period approximately 20% of methylene-labelled carbon was recovered as CO_2 . Closely related varieties susceptible to 2:4-D evolved only about 2% of the carboxyl carbon as CO_2 in a comparable period. Examination of a wide range of resistant and susceptible weed species showed that this difference in 2:4-D metabolism was not general.

In other collaborative work with Pomology, an apparatus was developed for exposing the leaves of apple to small quantities of $^{14}\text{CO}_2$. The uptake of CO_2 by detached leaves was not affected by previous treatment with 2-naphthaleneacetic acid. An examination was made of a radio tracer technique to follow the movement of products of photosynthesis in the tree (p. 56).

The behaviours of a further six ^{59}Fe -labelled chelates in calcareous soils were examined in collaboration with the Nutrition of Fruit Plants Section. This work has been extended to ascertain the fate in the plant of ^{14}C -labelled ethylenediamine tetraacetic acid and *cyclo*-hexane-1:2-diaminotetraacetic acid. Investigations with the A.R.C. Unit of Plant Nutrition of the distribution of ^{59}Fe in micro-organisms and leaves were continued.

Work was commenced* on the absorption of fungicides by cells using members of a homologous series of *n*-alkyl thioethers of 2:5-dimercapto-1:3:4-thiadiazole. The mineral oil/water partition coefficients of these compounds increased with increasing chain length to the amyl homologue and decreased for the higher members, an effect more marked when the compounds were almost completely un-ionized at low pH. Although the uptake of the homologues at equimolar concentrations by potato tissue was not correlated with alkyl chain length, maximum absorption occurred with the amyl member. Experimental evidence indicates that the uptake by potato discs is, at least partially, a cell metabolic process.

The uptake of the homologues by conidia of *Aspergillus niger*, *Helminthosporium sativum*, *Monolinia fructicola* and *Stemphylium sarcinaeforme* increased with increasing length of alkyl side chain to a maximum with the amyl homologue and decreased for the hexyl, heptyl and octyl members.

*During the tenure by Dr. Somers of a National Research Council of Canada Fellowship at the Science Service Laboratory, London, Ontario.

Experiments with *M. fruticicola* again indicated that the uptake was dependent on cell metabolism. No evidence was found that the compounds are physical toxicants, and it is suggested that their toxicity is due to chemical interaction with a lipoid component of the fungal cell and that this process is dependent on cell metabolic activity (p. 106).

Work has continued on the vibrating jet method for the determination of dynamic surface tension with the object of using the method for the examination of dilute aqueous solutions of surface active agents. Accurate measurement of the flow rate of a jet of liquid was made with a mains frequency timing assembly. Improved photographic images of the jet stream profile permitted the stream cross-section to be evaluated to within $1\ \mu$, giving a maximum error of 0.5% for a stream radius of 0.02 cm. This probably represents the limit of accuracy of the surface tension values obtainable by the method (p. 146).

Analytical and General Chemistry.

Work was continued on the nature of plant cuticles in investigations designed to give a better understanding of the behaviour and fate of spray deposits on plant surfaces, of the factors inducing phytotoxicity and of host-parasite relationships. Special attention was given to cutin, and a quantitative method for the determination of this component of isolated cuticular membranes was developed. Using this method, and the methods devised earlier for the waxes, the cuticles of leaves of cauliflower, cabbage, tomato, banana, strawberry, black currant, laurel and rhododendron were examined. Considerable variations were found in the levels of cuticle deposition and in the relative proportions of waxy constituents and cutin. The development of the cuticles of leaves and fruits of Cox and Worcester apple was also followed chemically throughout the season (p. 102). In collaboration with the East Malling Research Station an examination was made of the cuticles of Cox apple fruits grown under different nutritional conditions; this work is being continued and will be reported later. An investigation was made by the holder of the Boots Research Scholarship of the cuticles of leaves of species of *Euonymus* in relation to susceptibility to mildew infection.

An analytical method was devised for the determination of surface and internal phenolic compounds, expressed as phloridzin, in apple leaves. In preliminary work, the levels of the phenolic compounds in young and old leaves and the effect of different nutritional conditions upon their contents in leaves were examined. The leaching of phenolic materials from leaves by rain was also followed quantitatively.

Studies on the chemistry of dieldrin were continued with a view to developing a convenient colorimetric method for the determination of spray residues. The use of a complex of boron trifluoride and ether permitted the conversion of dieldrin to a ketone. A 2:4-dinitrophenyl-hydrazone was prepared from the ketone; the colour produced by interaction with tetraethyl-ammonium hydroxide was found to be stoichiometric (p. 95). Side-products were formed in the reaction and their structure is under examination. The use of boron trifluoride as the basis of a colorimetric method of assay of endrin residues was examined, but steric factors induced a much lower sensitivity.

Analytical work was carried out, in collaboration with other organizations, in relation to the use of insecticides and fungicides in the field. Coffee berries

from trials in East Africa conducted by the Coffee Berry Disease Research Unit were tested for mercury contamination (p.88); a preliminary investigation was made of the fixation of mercury by apple trees subjected to repeated applications of mercurial sprays; tobacco leaves from spraying trials abroad were examined for copper deposits, and DDT residues in soils were determined for the National Vegetable Research Station. The chemical assay of derris and lonchocarpus root was re-examined and an improved method proposed for the determination of rotenone (p. 99). Collaborative work on rotenone assay, including an assessment of the new method, has been carried out with other interested laboratories. Investigations on the determination of DDT and gamma-BHC in wheat flour were undertaken for the Analytical Panel of the Ministry of Agriculture's Scientific Subcommittee on toxic chemicals.

Plant Pathology.

A comparison of the efficacy of selected oxidised phenolic compounds from apple fruits in reducing the pectolytic effects of the enzymes of the brown rot fungus, *Sclerotinia fructigena*, showed that oxidised catechins and leucoanthocyanins were more effective inhibitors than oxidised chlorogenic acid. The production of polygalacturonase by germinating spores of *S. fructigena* was enhanced by the presence of asparagine, glutamine and ammonium salts.

In studies of the effect of nitrogen status of apple trees on susceptibility to fungus diseases, it was found that liability to canker (*Nectria galligena*) was associated with prolongation of summer growth and persistence of free amino-acids. In apples harvested from trees grown with different cover crop/manurial treatments, and inoculated with *Gloeosporium perennans*, there appeared to be a correlation between susceptibility to infection and the nitrogen status of the trees: fruits from nitrogen-deficient trees under grass rotted much more slowly than those from trees under clover or arable cultivation (p. 118).

Three applications of *n*-dodecylguanidine acetate (Cyprex) to Worcester Pearmain trees caused no phytotoxicity and gave a significantly better control of scab than captan applied at the same dates. The results of the 3-year comparison between captan and lime-sulphur spraying of Cox at Long Ashton have been published: on Type II rootstock yields from the captan-sprayed plots were 40% above those from the lime-sulphur plots (9). In a parallel experiment at Luddington there was no such difference because of the depressing effect of mildew damage on the captan-sprayed trees.

Leader tipping of Cox trees at Long Ashton in January halved the mildew infection in June in comparison with that on trees receiving renewal pruning; secondary infections were better controlled by lime sulphur than by Karathane. At Luddington, treatment with DNC in March led to a 60% reduction in the outbreaks of primary mildew in May.

The ability of *Gloeosporium perennans* to infect apple shoots through leaf scars was demonstrated. Tip-pruned extension shoots were shown to be susceptible to *Gloeosporium* attack throughout the summer; such summer-induced infections often showed renewed spread in the following spring. Summer infections contributed little to total spore output: November-initiated cankers provided over 90% of the conidial inoculum.

Ascospore production on overwintered black currant leaves infected with leaf spot (*Pseudopeziza ribis*) was inhibited by dipping the leaves in 0.1%

sodium pentachlorophenate or DNC. Spread of the disease during the summer was checked by pre-cropping and post-cropping sprays of either captan or zineb.

In the Luddington trial against *Botrytis* rot of strawberries, thiram gave significantly better control than captan. No clear evidence of tainting of the processed fruit was obtained.

On Leveller gooseberries at Efford, the mean percentages of mildewed fruit in the sprayed plots on 30th July were : Karathane, 3.1 ; washing soda, 4.7 ; control, 13.9. These differences were not maintained in the shoot infections, assessed a month later.

In experiments made in collaboration with Organic Chemistry on the relations between chemical constitution and fungicidal activity it was found that the activity of a number of compounds, including captan, appeared to be related to their ability to accumulate within the fungus spore.

Preliminary tests of the fungistatic value of selected mineral oils showed that applications of oil emulsions did not prevent ascospore discharge but checked mycelial growth and reduced the water-dissemination of spores from conidial pustules (p. 131).

Entomology.

Biology of Insect Pests.

To determine the damage caused to fruit trusses of Worcester Pearmain apple by known populations of the Oat Apple aphid (*Rhopalosiphum insertum* Wlk.) and the Rosy Apple aphid (*Sappaphis plantaginea* Pass.), infestations of first instar nymphs were made to uncaged flower trusses, the number per truss varying from 1–20. During the transfer, many of these first instar nymphs suffered severely from parasites and predators. These preliminary observations suggested that no injury to fruit resulted from populations of *R. insertum*, but parthenocarpic fruit was sometimes formed where one to three fundatrigeniae of *S. plantaginea* were present on a truss.

A severe attack by the Red-legged weevil (*Otiorrhynchus clavipes* Boisd.) occurred on a block of black currants that had been strawed for several seasons. Up to 250 weevils per bush were found in some areas of the plot, and as the currants were growing slowly at the time they suffered severe damage. Control was effected with DDT emulsion at a concentration of 1 lb. DDT/100 gallons applied by automatic sprayer at 300 gallons/acre. Parthenogenicity occurs in these weevils, and of those collected 98.6% were females. In the laboratory egg laying occurred from May 14th–October 9th ; the average number of eggs laid by mated females was about 1,750 and by unmated females about 750 ; the initial hatch of eggs was over 90%.

Associated with this infestation was the Clay-coloured weevil (*Otiorrhynchus picipes* F.) which emerged slightly later and in smaller numbers. No males of this species were observed, and only a very few eggs were laid in captivity.

The biology of the Big Bud Mite is under re-investigation as a basis for further work on control measures and on the transmission of the virus causing reversion. A method has been evolved for the rapid assay of mites within a bud, which overcomes the difficulties associated with the normal visual diagnosis by the size of the bud. Laboratory experiments indicate that wind transmission of the mites is extremely rare. Alternative vectors for reversion are under investigation.

In collaboration with the Willows Section work on the biology and control of the Willow weevil, *Cryptorrhynchus lapathi* (L), was started. The adult weevil feeds by puncturing the willow rods and causes two types of damage : misshapen rods due to damage to the growing point when the rods are 12-18" high, and rods deformed later by punctures near their base. The larval life is spent in the stool and may extend over two years. Laboratory experiments on the control of the adult weevil showed that dieldrin, BHC and Thiodan, at rates of 4 and 16 oz./acre, controlled the adult weevil, but DDT was ineffective even at $2\frac{1}{2}$ lb./acre.

Regular observations of the Leaf Hoppers attacking the cider apples Sweet Alford and Stoke Red and the dessert apple Laxton's Superb were made on samples of 40 leaves collected from each tree in a plot at Long Ashton. The first hopper nymphs were noted on May 28th ; many died, probably because of the residual effect of a BHC-DDT-lime sulphur-succinate spray applied on May 13th. The species recorded were *Erythroneura* sp. (probably *alneti*), *Typhlocyba debilis*, *T. froggatti* and *T. rosae*. A comparison of the number of nymphal forms with the alate population on Sweet Alford suggested that some of the adults had flown in from an outside source. At the beginning of the season the population consisted mainly of *Erythroneura* sp. ; *T. debilis* and *T. froggatti* occurred in much smaller numbers, and there were very few *T. rosae*, which did not appear until the end of August. It appears that *Erythroneura* has one generation whereas *T. debilis* and *T. froggatti* have two (p. 139).

Bio-assays of insecticides and fungicides.

The effect of DDT-type compounds on the respiration of ganglia and muscle tissue of the cockroach (*Periplaneta americana*) has been investigated. The typical symptoms of DDT poisoning clearly demonstrate an effect on the neuromuscular system and follow the sequence (1) an increasing general tremulousness ; (2) ataxic gait and hyperactivity ; (3) paralysis, accompanied by tremors which slowly disappear, and (4) death. The respiration rate of the insect (measured as oxygen uptake) is related to the activity at various stages of poisoning, rising from 200 μ l./hr./g., for the normal insect, to between 2,000-3,000 μ l./hr./g., for the hyperactive stage, and then slowly falling to about 200 μ l./hr./g., just before death. Muscle tissue from poisoned insects, at various stages, showed no change in the respiration rate ; it remained at about 800 μ l./hr./g. The respiration of thoracic ganglia, however, showed a change of rate similar to that of the poisoned adult, rising from 1,600 μ l./hr./g., to 3,000 μ l./hr./g. and then falling to 1,000 μ l./hr./g. just before death. The increased respiration rate of the ganglia is correlated with the hyperactivity due to the action of DDT, and may be caused either by an accumulated oxygen-debt in the ganglia, due to intense nervous activity, or by an increased activity of neurons which continues even when the ganglia are removed from the insect. The work is being continued with insecticides other than DDT-type compounds.

At times a single application of a spring wash fails to provide a satisfactory control of the apple aphids and Winter Moth (*Operopthera brumata*). Observations on the behaviour of the insects under laboratory conditions after hatching showed that the failures were mostly due to uneven distribution of the spray,

particularly in seasons when the egg hatch occurred over a period of 7-10 days. All stages of the aphid nymphs and moth larvae were killed by direct contact action with "full coverage" sprays of either 0.025% DDT or malathion. The residual action of the malathion spray on leaves gave a complete control of aphids wandering on the surface for three days after application, but failed to control any instar of the moth larvae. Aphids and moth larvae that had entered flower buds before spraying were frequently able to escape contact with DDT, BHC and malathion, regardless of concentration when applied as discrete droplets. Malathion, applied as a spray of high wetting properties in liberal volumes, again proved a valuable spray for controlling severe aphid attacks on the foliage of a wide range of fruit crops.

Laboratory applications of 0.01% and 0.04% malathion with succinate wetter (0.025%) by large and small volume methods to well-established woolly aphid colonies failed to give control. Large volume applications of the higher concentration gave the best initial kill but the colonies were re-established after 2-3 days. Prolonged hand lance spraying of colonies in the field also failed to control this insect and showed that malathion at these concentrations is effective only against the insects on the surface of the colony.

Spray Techniques.

Work has continued on the phytotoxicity of surface-active agents used as spray supplements. The results obtained on apple and plum leaves during the past four seasons show that surface-active materials containing a branched alkyl chain in the hydrophobic portion of their molecule are better wetters than corresponding materials containing a straight alkyl chain. In any homologous series of materials, wetting ability increases with increase in size of the alkyl group. With the non-ionic ethylene oxide condensates, maximum wetting occurs with those materials containing the minimum number of ethylene oxide groups that confers water solubility.

Phytotoxicity appears to be governed by the nature of the ionic charge, by the physical size of the molecules or ions and to a lesser extent by the nature of the gegenion. All non-ionic materials cause little or no damage on apples and plums; anionic and cationic materials cause variable damage depending on their chemical structure and on the nature of the leaf surface. In any homologous series of surface-active agents the phytotoxicity appears to pass through a maximum as the molecular size is progressively increased. Since this maximum usually occurs at fairly short alkyl chain lengths (dodecyl or below), for most practical purposes phytotoxicity decreases as the size of the alkyl group increases.

Further work has been carried out on the nature of the possible reactions taking place between surface-active agents and leaf surfaces. Immersion of leaves in solutions of cationic surface-active agents may produce either activation or inactivation of the solution, effects which are directly governed by the chemical nature of the surface-active material employed.

Small-scale field experiments to compare the effects of large and small volume spray applications of DDT/BHC emulsions on the control of apple aphids at the green cluster stage were continued. It was again shown that complete control was obtained by large volume application (full coverage) of the dilute materials (0.025% DDT, 0.006% gamma BHC) with 0.025% nonyl

sodium sulpho-succinate wetter. Inferior control was obtained when the wetter was omitted. Small volume "mist blower" applications of the same dilute materials gave a poor control, though the infestation was reduced when the wetter was included. An increase in concentration of the insecticides to ten times (0.25% DDT, 0.06% gamma BHC) failed to give a complete control. The indications are that aphid control with DDT/BHC emulsions at this stage depends not upon the concentration of insecticides but upon their deposition at the correct site on the flower buds. Large volume spraying is, at the moment, the most reliable method of application for this purpose.

Preliminary investigations have indicated the value of fluorescent materials for the tracing of spray liquid deposition. Field trials using a fluorescent tracer have shown that a rapid qualitative comparison can be made of the spray cover obtained by hand lance, vertical mast and overhead boom spraying of black currants. While the most consistent results were obtained from the hand lance spraying those from the vertical mast spraying were almost as good. Inferior cover resulted from the horizontal boom spraying except on the top surfaces of the leaves, but cover was greatly improved by adjusting the spray nozzles to an angle of 45° and spraying the rows in two opposite directions.

Fluorescent tracers were also used in studying in North Borneo the control of the aphid *Pentalonia nigronervosa*, the vector of Bunchy Top virus of Abaca hemp. This work, which has been followed by extensive field trials, is described on p. 142. It was found essential to wet thoroughly the aphids living under the overlapping portions of the sheathed leaves.

A large number of DDT assays were made by the Chemistry Section on samples sent by air from Kenya and the Solomon Islands, where further work was carried out on the control of Nutfall Bugs of coconuts. The most satisfactory control was obtained by contact action of a high concentration of DDT applied in oil. Chemical control is not feasible in the Solomon Islands but the investigations in Kenya continue.

The levels and distribution of copper deposits have been studied during work on the control of Frog-eye of cigar-wrapper tobacco in Borneo.

Spray Machinery.

Work has continued on the internal mixing, high pressure compressed air nozzle (1957 Report) which aims at producing a closer spectrum of droplets without the need for small apertures or fine adjustments; the nozzles have been operated by a tractor-mounted and driven rotary drum compressor.

Further experiments were made in the development of an air nozzle suitable for operation with a fan of 2,000 cu. ft. per minute at pressures up to 2 lb./sq. in. and capable of producing a wide range of throughputs of liquid of relatively small droplet sizes. The nozzle has been incorporated in a machine that may prove suitable for spraying bush crops such as coffee, currants, and dwarf pyramid apples (p. 152).

Small power, hydraulic sprayers have been made for experimental work in North Borneo and Kenya and pneumatic sprayers with lances of Long Ashton design have been supplied to North Borneo for use by Chinese smallholders. A lattice steel mast, capable of extension to 70 ft., has been erected for studies of the distribution of spray droplets.

Cider and Fruit Juices.

Organic Components.

Work on the nitrogen content and free amino acids of a range of soft fruits has been reported (5) and is in course of publication.

Investigation of the protein of apple juice has been continued mainly by use of dialysis to eliminate soluble solids. Exhaustive dialysis of a juice at 0°C resulted in extensive oxidation of phenolic constituents and much brown precipitate was formed. The dialysed juice retained about half of its original tannin content. The deposit consisted chiefly of polyphenols with only 1.3% protein in the dry matter; nevertheless more than half the non-dialysable nitrogen was precipitated in this way.

Dialysis against 100 p.p.m. SO_2 solution largely prevented oxidation. The brown opalescent suspension, obtained by concentrating to 1/100th of the original volume, contained 12.2% protein in the dry matter. Chromatograms of the amino acids obtained by acid hydrolysis showed the usual pattern for protein and were similar to those reported by Hulme for protein from immature Bramley's Seedling apples.

Attempts were made to separate the protein from associated impurities (polyphenols, pectin and polysaccharides). Fractionation with ammonium sulphate, fractional elution from the resin IRC.50, and adsorption on calcium phosphate all failed to separate protein from polyphenols. It was concluded that to isolate protein from apple juice, polyphenols must be removed at an early stage under reducing conditions.

Treatment with zinc chloride in 20% ethanol solution at pH 5.8 has been found to precipitate almost all the protein (and pectin) from apple juice.

Investigation of the keto-acids in juices and ciders was continued. Ion-exchange resin and silica gel column techniques are not easily applicable to keto-acids owing to decomposition or polymerisation during concentration of the juices or ciders or of the acid fractions. Keto-acids have therefore been investigated after preliminary conversion to their 2:4-dinitrophenylhydrazones, which have been purified on alumina columns and tentatively identified by paper chromatography. Catalytic reduction with palladium-barium sulphate to the corresponding amino-acid was a further aid to identification.

Juices contained only trace amounts of keto-acids: α -ketoglutaric, pyruvic, glyoxylic and oxaloacetic acids have been tentatively identified. Considerable amounts of keto-acids were formed during both natural and sulphited fermentations. Much larger amounts were formed in fermentations of high pH juices than of low pH juices. The main keto-acid formed was pyruvic acid with small amounts of α -ketoglutaric acid. At a later stage of fermentation the amount of keto-acid decreased, particularly in fermentations by the natural flora. In sulphited fermentations reduction products of keto-acids have been found, namely malic acid, sometimes in large amounts, and small or trace amounts of lactic and α -hydroxyglutaric acid.

While 2-methyl-2:3-dihydroxybutyric acid was sometimes present in trace amounts in juices (43), this acid was formed during the fermentation of sulphited juices together with 2:3-dihydroxyisovaleric acid and 2-ethyl-2:3-dihydroxybutyric acid. Benzoic acid and fumaric acid have been isolated from sulphited fermentations and 2-keto-gluconic acid has been tentatively identified. Mucic acid has been isolated from both juices and ciders.

A study of the neutral carbonyl compounds has shown acetaldehyde to be a minor component of both juices and ciders: the higher members occur in larger amounts.

Pectin Enzymes.

Modifications in cider-making procedures, notably the increasing use of concentrated juices, have given rise to problems in clarification which have called for a re-investigation of pectin changes during fermentation.

It has been found that, in fermentations carried out by the traditional method, pectin is broken down by the combined action of pectin methylesterase derived from the fruit and a polygalacturonase produced by yeasts present in the natural juice flora. The latter enzyme does not appreciably attack fully methylated pectin, but splits partially de-esterified pectins to a range of lower galacturonides. In heated juices or concentrates, where pectin esterase is absent, the original pectin remains intact throughout fermentation. Conversely, fermentation of a sterile juice by an added yeast will again leave the pectin undegraded if the yeast is not a producer of polygalacturonase; in such fermentations the addition of pectin enzymes may be necessary. An enzyme-producing *Saccharomyces* species isolated from cider has been found to produce an endopolygalacturonase apparently identical in properties with that produced by *S. fragilis*, but the amount of enzyme produced is less.

The use of yeasts as sources of polygalacturonase enzymes has been considered, and enzyme preparations from *S. fragilis* have been found effective in the depectinising of black currant and other fruit pulps in juice extraction. The yeast enzyme acts in conjunction with the fruit pectin methyl-esterase.

Attention has been further directed to the causes of variation in the efficiency of pectin degrading enzymes in fruit systems. Fruit phenolics, especially a leuco-anthocyanin, have been found partially to inhibit a range of pectic enzymes including methylesterase, yeast polygalacturonase and commercial enzyme preparations (35). The inhibitors restrict the rate of pectin degradation as measured by viscosity change of chain splitting. The results confirm work (p. 34) on the inhibition by fruit phenolics of the pectic enzymes secreted by certain plant pathogens.

Microbiology.

The titratable acidities of sulphited low acid juices increase during fermentation because of the formation of succinic and pyruvic acids and of malic acid, which in the absence of sulphite is converted by bacterial action to lactic acid. From a series of sulphited ciders, 100 yeasts were isolated, 44 of which, belonging to the genus *Saccharomyces*, were able to synthesise malic acid when inoculated into sterile, low-acid apple juice. The production of malic acid was more rapid at 25°C than at 15°C, the normal fermentation temperature, and occurred after a drop of approximately 15° in Sp. Gr., the period of greatest yeast growth, but not following inoculation of a sterile-filtered cider previously fermented to the same stage with *S. uvarum*. Traces of malic acid have now been produced in preliminary trials with synthetic media.

The bacterial flora of ciders made from diluted apple concentrates of commercial origin was examined in controlled laboratory fermentations and

compared with the flora of ciders made from the same concentrates in the factory. The diluted concentrates contained very few bacteria, but the ciders produced in the factory were heavily contaminated with acetic acid bacteria derived from the wooden fermentation vessels.

A series of ciders was canned and bottled and their flavours compared after 6-months storage. Contrary to results obtained in cider-factories, the canned ciders were better than the bottled, and there was no evidence of any harmful effect of sulphur dioxide in the cider. The experiments are being repeated on a wider range of ciders.

Further examination of acetic acid bacteria in juices and ciders has confirmed that these organisms survive throughout fermentation and subsequent storage. In comparative fermentations of the same low-acid juice, either in carbon dioxide or in air, the acetic acid bacteria were able not only to survive but to increase. The bacteria increased in number during periods of least fermentative activity, that is when the yeasts were reproducing prior to fermentation, and later when fermentation had ceased. While the underlying pattern of bacterial growth was similar for both fermentations, the numerical increase of those in the fermentation exposed to air was considerably greater than that observed in the fermentation kept under a constant atmosphere of carbon dioxide (p. 160). The persistence of these bacteria under relatively anaerobic conditions emphasises the need for microbiological control throughout the whole process of cider-making.

The most commonly occurring acetic acid bacteria were those hitherto referred to as the Suboxydans group of the genus *Acetobacter*, but now thought to constitute a new genus with the proposed name *Acetomonas*. One particular juice made from a batch of Stoke Red apples contained only species of *Acetomonas* throughout fermentation and storage. In general the *Acetobacter* species appeared during the later stages of fermentation and in storage; the three species most frequently encountered were *A. xylinum*, *A. mesoxydans* and *A. rancens*, all of which are known to interchange freely from one species to another by mutation.

Several new species were encountered, amongst them an organism that has been given the name *Acetobacter estunense* (11). This resembles the established species *A. lovaniense* except that it produces a polysaccharide which gives a positive cellulose reaction with iodine and sulphuric acid. Another organism, resembling *A. aceti*, was isolated; this also produces a substance similar in appearance to, and giving the same positive cellulose reaction as, *A. estunense* (12). The 'cellulose' produced by these two organisms is, however, soft and slimy in contrast to the tough leathery growth of the well-known cellulose-producing species *A. xylinum*. The discovery of pseudo-cellulose production by these *Acetobacter* species had made it easier to correlate some of the original descriptions of these organisms with the present-day system of classification (13).

Metallic Complexes.

The non-enzymic oxidation of catechol and other phenolic compounds in the presence of cupric ions has been studied using the Warburg technique. Oxygen consumption varied according to the nature of the buffer employed, decreasing in the order acetate, malate, citrate in weakly acid solution, and in the order tris-maleate, phosphate in neutral solution. The cupric catechol

complexes were oxidised in air, and addition of buffers at the same pH reduced the oxygen uptake. Oxidation of catechol by copper was inhibited by Neo-cuproine, suggesting the formation of cuprous ion as an intermediate in the reaction, while benzene sulphinate increased oxidation. The oxidation of a number of phenolic compounds in the presence of cupric ions at pH 5.6 (acetate) decreased in the following order: homo-catechol, caffeic acid, *d*-catechin, hydroquinone, catechol, chlorogenic acid, protocatechuic acid.

The suggestion that pre-harvest copper sprays be used to control Leaf Spot in black currants has raised the question of the effect of copper on the stability of vitamin C (ascorbic acid) in black currant juices. Accordingly, the oxidation and losses of ascorbic acid that can occur in model systems and juices are being investigated using the Warburg technique. In citrate buffer model systems, corresponding in composition and pH to black currant juice, the oxidation of ascorbic acid in the presence of copper was considerably increased by the addition of very small amounts of iron, although iron alone had little effect. Of juice components examined, black currant anthocyanins greatly increased ascorbic acid loss while glucose considerably reduced losses. In model systems at juice pH, ascorbic acid losses in the presence of copper, with and without iron, increased according to the buffer used, in the order malonate, citrate, malate, quinate, formate. This order is that of increasing free cupric ion concentration and indicates an appreciable measure of copper complexing by citrate. Oxidation in model systems generally paralleled ascorbic acid losses, but the number of oxygen atoms consumed per mole of ascorbic acid oxidised varied considerably with the nature of the components present.

The addition of copper salts to black currant juices increased the rate of loss of ascorbic acid, the effect of copper being most pronounced at very low concentrations. The addition of iron salts to juices had little effect. The addition of Versene or Neo-cuproine to juice reduced oxidation to very small amounts. It seems likely that a large part of the oxidation of ascorbic acid in an uncontaminated juice in the presence of air may be attributed to the natural copper present, the effect being enhanced by iron naturally present. The results suggest that contamination of juice by even small amounts of copper would increase ascorbic acid oxidation in the presence of air.

Domestic Food Preservation.

To obtain more information on the comparative nutritional quantities of vegetables preserved by canning and by freezing under domestic conditions, ascorbic acid determinations were made on 50 varieties when freshly picked and when scalded prior to preserving; further determinations will be made after thawing and after cooking, for comparison with canned products stored for the same length of time. Data for loss of ascorbic acid are already available for 26 varieties canned in 1957.

An examination of the bacterial flora of different kinds of vegetables is being made prior to freezing and after storage at 0°F. for periods up to 6 months; the growth of the main organisms, particularly pathogens, will then be followed under different storage conditions.

The effects of methods of preparation on the quality of some varieties of vegetables have been further tested; some results are given at p. 175.

A bulletin on domestic meat and poultry preservation by bottling, canning and freezing, was published by the Central Office of Information in

April (18). Accounts of the microbiological and heat-penetration studies needed to ensure the safety of the recommended methods have been published (19,20). Similar work is now being carried out on the heat-resistance of bacterial spores in different kinds of vegetables and on heat-penetration rates in different packs of bottled or canned vegetables.

The quality assessment of fruit varieties, preserved by different methods, has been continued. Over 70 varieties have been preserved during the year; a note on some gooseberry varieties is given in this Report (p. 179). A list of strawberry varieties suitable for canning, freezing and jam-making has been prepared for a revised edition of the Ministry's Strawberry Bulletin No. 95.

Acidified sugar syrups containing the fungicides captan, thiram and Karathane, at concentrations of 4 to 20 p.p.m. were canned in plain and fruit lacquered cans and stored at 80°F. After 6 months both thiram and captan caused marked corrosion of the cans, and were easily detected by a tasting panel. Karathane appeared to have no adverse effect on the cans and could not be detected in the concentration used. In further tests smaller concentrations of thiram and captan are being used and glass containers are being included to see whether taint is partly caused by the metal of the cans. Tasting trials of fresh and preserved strawberries treated with these fungicides, and of black currants sprayed with malathion and BHC (lindane) have so far given less conclusive results.

Further tests have been made of the suitability of covers for pickles and jam (see p. 181), and of containers for frozen foods.

Willows

Variety Collection.

Cuttings of seven varieties were received, including three specially selected fast growing hybrids from Germany, bringing the total number of varieties in the collection to 189. The cuttings of *Salix aquatica gigantea*, Clone 56, received in 1957 have proved to belong to two distinct clonal stocks.

Experimental Work.

In further studies of the root pattern of basket willows in relation to possible damage by mechanical cultivation, measurements were again made on a plot planted in 1954. The proportion of roots at a depth of 0-3 in. was 30.9%, compared with 23.1% in 1957; and 30.9% at 3-6 in., compared with 32.1% in 1957; the proportion in the surface six inches has risen slightly from 54.5% in 1956 to 61.9% in 1958. The mean length of the longest root per stool had increased to 90 in.; its mean depth below the surface was 6 in.

On the plots which received inter-row ground sprays of 2:4-D (3,700 p.p.m.) and 2:4:5-TB (3,900 p.p.m.) in 1957 the ground cover in 1958 consisted entirely of Greater Bindweed. 2:4-D effected a better control than 2:4:5-TB, the relative surface occupied by bindweed in plots receiving 2:4-D, 2:4:5-TB and controls being 15%, 30% and 50% on June 4th and 60%, 100% and 100% on July 17th respectively. On plots which received foliar sprays of 2:4:5-TB in 1957 concentrations of 1,000 and 1,500 p.p.m. had completely controlled bindweed whereas 500 p.p.m. had not; in the last plots it was present as 10% of the vegetation. No stools were killed by these treatments but the vigour of this year's growth was reduced.

In collaboration with the N.A.A.S., experiments were made on the control of willow rust, *Melampsora amygdalina*, in a heavily infested bed. Attempts were made to kill teleutospores in the leaf litter by applications of DNC (450 gall./acre at 0.1%) in April and tar oil (150 gall./acre of 15% strength) in May. DNC had no significant effect on the length and number of shoots produced, but the shoots from plants treated with tar oil were 12" shorter than those of the controls at the end of July. The effect of spraying the developing shoots with copper oxychloride (1.8 lb./acre) and phenylmercuric chloride (0.06 lb./acre) in May and June could not be assessed, as no stem cankers appeared on the experimental plots throughout the season; by September there was, however, a heavy (96%) uniform rust infection of the leaves of both treated and control shoots.

Preliminary studies of the Willow weevil, *Cryptorhynchus lapathi*, are described under Entomology (p. 36).

An important problem encountered in processing basket willows is the shortness of the period during which they can be peeled to produce white rods. The most successful of 9 treatments showed that the peelable condition could be advanced 10 weeks by exposing the cut rods to a temperature of 37°F. for 5 weeks, followed by 3 weeks at 60°F. with a day length of 16 hours.

Advisory Work.

There were 220 requests for information during the year. 68 visits to growers were made as below :

	South West	Home Counties	East Anglia	Mid- lands	North	Wales	Scot- land	Foreign
No. of enquiries	66	24	63	13	21	6	12	15
No. of visits	44	3	16	3	—	—	—	2

Three quarters of the enquiries were concerned with basket willows and one quarter with bat willows.

Of 14 requests for planting material from research and educational centres, the most important was for a total of 8,000 cuttings of 20 varieties for a trial amenity scheme in Lancashire. The cuttings have grown well and have proved very suitable, combining quick growth and decorative bushy habit with cheapness, ease of planting and tolerance to mutilation. Further cuttings are to be supplied in 1959. Representatives of the complete variety collection were sent for taxonomic purposes to Northumberland, and in exchange 30 new varieties are promised for planting in 1959. 16,000 cuttings were supplied to the Prison Commission to complete their planting in Suffolk; the overall growth made in 1957 and 1958 has been good, but poor patches occur in areas of impeded drainage.

There was again in 1958 an increased import of foreign willows which, coupled with a reduced demand for baskets, made it difficult for growers to dispose of their crop. In consequence prices at the Somerset auctions averaged only £40 per acre, compared with £68 in 1957 and £118 in 1956. In contrast to 1957 there was a low incidence of both willow rust (*Melampsora amygdalina*) and the Willow weevil (*Cryptorhynchus lapathi*) but the Willow Bean-gall sawfly (*Pontania gallicola*), last noted in 1954, was locally abundant and damaged the end-of-season growth.

The survey of the Somerset willow-growing area has been extended to obtain information on productivity. Of 1,326 acres now in production, 396 acres were planted in 1953-58, 174 acres in 1948-53, 333 acres in 1938-48, 384 acres in 1918-38, and 39 acres prior to 1918. Black Maul (now 984 acres) and Champion Rod (167 acres) have throughout been the most popular varieties.

Following the successful trials of cricket bat waste as pulping material (1957 Report) contractors have been quick to use this market for their hitherto unwanted produce. *Salix aquatica gigantea*, Clone 56, has grown better this year and sufficient material will shortly be available to find whether it will prove economic for pulp production in this country. Poplar is preferred to willow as a pulping material, but willows can be established where poplars will not grow.

Two local species of willow and six hardy selections from the Long Ashton collection are being tried experimentally for the improvement of heavy clay soils at exposed sites in N.W. Devon where conifers have not been successful.

Statistics.

Experimental Design and Analysis.

Assistance has again been given in almost all branches of the Station's research activities, as well as to other members of the University. Several short-term trials, with both old trees and pyramids, were designed and analysed for the 1958 season, as were a number of greenhouse and laboratory trials.

Sites outside the Station have been examined for the design of long-term pomological trials with cider varieties and with virus-infected material; one site in Gloucestershire and one in Somerset are being planted up in the current season. A trial of this type has also been designed for Plot 22 at Long Ashton.

Trials carried out at Experimental Horticulture Stations, especially nutrition trials, have yielded a considerable amount of data requiring analysis, mostly of the straightforward factorial type with no more than a simple covariance correction for missing plants within plots. Long-term trials with top fruits are beginning to yield results; these are also designed factorially with split-plot arrangements to include cover crops. Experience has been gained, from data obtained on Plot 18 at Long Ashton, in the analysis of a split-plot layout where observations from a number of plots are completely missing; existing published work does not treat this problem completely, and it is hoped to clarify some aspects with the aid of suitable practical data.

Recent developments in the conduct of multi-factor experiments have suggested that the classical factorial approach may with advantage be modified to calculate "response surfaces" relating the yield of a crop to the levels of fertilizers applied. A pot experiment in strawberry nutrition has been designed to enable the two methods of analysis and interpretation to be compared in 1959, and the crop results are awaited with considerable interest.

In the study of droplet shapes, efforts have been directed towards the expression of the equations of the several different shapes in a general two-parameter family, to which goodness-of-fit may be tested with the aid of computing machinery. Equations in polar co-ordinates appear most suited to this requirement.

Sampling systems appropriate to various investigations have again been evolved, for the study of both pathological and pomological characteristics.

Biometric Studies.

A report on the trial of captan and lime sulphur as fungicides against apple scab, made in collaboration with Pathology, has been published (9).

Tasting-panel assessments of samples of fruit from pathological field trials have continued. Sensitivity of detection of taints has again varied considerably; hence the various methods of assessment have been examined using a series of prepared solutions, consisting of artificial flavour bases with varying amounts of the appropriate fungicides added. As expected, the "triangular" method of tasting has been the only one to give really satisfactory results.

The assessment of black currants for leaf spot has been carried out on a wide range of varieties for a complete season, to enable both rates of increase and times of onset to be compared.

In collaboration with the Chemistry Section, a method is being sought for calculating the volumes and surface areas of apples and tomatoes from measurements which are simple to obtain, such as heights and diameters.

AGRICULTURAL RESEARCH COUNCIL UNIT OF PLANT NUTRITION (MICRONUTRIENTS)

Plant Physiology.

Molybdenum Nutrition.

A large number of antimetabolites known to interfere with the synthesis of proteins and nucleic acids have been tested for their ability to inhibit the synthesis of nitrate reductase in higher plants. So far only actidione, 1:2-dichloro-4-(*p*-nitrobenzene sulphonylamido)-5-nitrobenzene (DCDNS) and polymixin B inhibited enzyme synthesis when infiltrated with nitrate or molybdenum into leaf tissues of cauliflower or mustard. The presence of nitrate had a stabilising effect on the enzyme *in vivo*.

An unusually high nitrate reductase activity was found in young leaves of vegetable marrow; inhibitory factors were present in extracts of cotyledons, their effect increasing with the age of the plant. Root extracts contained a nitrate reducing enzyme that appeared to differ, in substrate affinities and solubility, from that in the leaves.

In a study of the effects of molybdenum supply on the growth patterns of cauliflower grown with nitrate in water culture, molybdenum deficiency was found to check leaf expansion, dry weight increase and chlorophyll content within two weeks of germination; to reduce net assimilation rate, relative growth rate and leaf area within two to three weeks, and to decrease the rate of formation of leaf primordia within three weeks of germination. Similar effects occurred with mustard about one week later than with cauliflower.

Histological studies with cauliflower have shown collapse of the leaf epidermis to be a consistent effect in the production of chlorotic lesions in the early stages of whiptail.

Phosphorus Metabolism.

The fractionation of the principal phosphorus components in leaves (1957 Report) was applied to cauliflower, lettuce, tomato, mustard and vegetable marrow grown with nitrate or ammonia nitrogen at normal and deficiency levels of molybdenum. In marrow and tomato, organic phosphorus was relatively high in the alcohol soluble and ribose-nucleic acid (RNA) fractions; acid-labile phosphorus occurred mainly in the RNA fraction. In cauliflower the organic phosphorus was greatest in the alcohol-soluble fraction and acid-labile phosphorus was greatest in the 0.1 N HCl acid-soluble and deoxyribose-nucleic acid fractions. The effects of molybdenum deficiency were neither simple nor consistent; there were usually decreases in organic phosphorus in most fractions.

Acid phosphatase activity was assayed in leaf extracts of tomato plants grown in complete nutrient and in nutrients lacking phosphorus, potassium, magnesium, iron, manganese, copper, zinc, boron or molybdenum; nitrogen was supplied as nitrate, nitrite or ammonia. Activity was very high with phosphorus and zinc deficiencies with all nitrogen sources and with copper deficiency in the presence of nitrite. Activities were also increased markedly by deficiencies of potassium and boron and were generally higher with nitrite than with nitrate. Molybdenum consistently increased phosphatase activity, on a protein basis, in extracts of cauliflower, tomato, sunflower, mustard, lettuce, spinach, beet and lucerne, grown with nitrate.

Tissue Culture.

In work on excised roots in aseptic cultures, using seeds of tomato as a source of root-tip inocula, the effects of deficiencies of iron, manganese, copper, zinc, molybdenum and iodine were assessed by measuring fresh weight, lengths of main axis and lateral roots and relative numbers of cells per culture. Iron deficiency resulted in an almost complete cessation of cell division after four to five days; no lateral primordia grew out and the main axis lengthened mostly by cell elongation. Growth was resumed if the roots were transferred to a medium containing iron within the next five days. Manganese deficiency only partially suppressed cell division in the first passage and division continued in the lateral meristems after the apex was finally killed. The effects of copper deficiency appeared first in the suppression of lateral meristems and later in the apex, whereas with zinc deficiency the apex was first affected and lateral root growth continued after the apex had ceased growth. Symptoms of molybdenum deficiency appeared gradually; the growth of both apex and laterals was affected, very few roots surviving the third 7-day passage in a deficient medium. Iodine deficiency caused a marked decrease in growth but did not kill the root.

Two forms of rotary culture apparatus were used successfully in growing tissue masses from explants of carrot phloem. The demonstration of symptoms of micronutrient deficiency was prevented by the need to use large quantities of coconut milk, for which a combination of casein hydrolysate and 1:3-diphenylurea was found an inadequate substitute. A number of cultures of stem tissue, some of which require no external growth factors for proliferation, have been received from Professor Gautheret and Dr. Heller of the Sorbonne; they are being subcultured to obtain a stock of material for experiment.

Nutrition of Micro-Organisms.

Nitrogen Metabolism.

Nitrate reductase. A study of the factors affecting the formation of nitrate reductase in mutants of *Neurospora crassa* has shown that several amino acids inhibit enzyme formation and that ammonia is toxic *in vivo* but not *in vitro*.

Nitrite reductase. Acetone powders of *Neurospora* felts were found to show nitrite reductase activity. The addition *in vitro* of iron to cell-free extracts prepared from iron-deficient felts and of copper to those from copper-deficient felts reactivated the enzyme, confirming the previous finding (1957 Report) that these two metals are required for enzyme activity.

Hydroxylamine reductase. This enzyme has been reported by other workers to catalyze reversibly the production of ammonia from hydroxylamine in *Bacillus subtilis* and in *Aspergillus flavus*. In experiments made with cell-free extracts of both organisms, no production of hydroxylamine from ammonia could be detected; this agrees with the results obtained with the *Neurospora* enzyme. At pH 8, an apparent reduction of DPN occurred in the presence of ammonia, without a concomitant production of hydroxylamine.

Glutamic dehydrogenase. Active preparations of this enzyme were made from acetone powders of felts of *Neurospora* by extracting them in glycine-NaOH buffer at the optimum pH of 8.5. The enzyme can utilize either DPNH or TPNH as an electron donor; with DPNH the dissociation constant (K_m) for the enzyme-substrate complex was determined as 7.5×10^{-2} moles α -keto-glutarate/litre. The enzyme had a specific requirement for α -keto-glutarate: other keto acids such as oxalacetate and oxalosuccinate were not utilized. Its activity was markedly depressed in zinc-deficient felts and restored by adding zinc *in vivo*. In mutants of *Neurospora* requiring pyridoxine or riboflavin the activity of the enzyme was not reduced by the omission of either vitamin.

Denitrifying bacteria. Stoichiometric studies of the production of nitrogen by whole cells of *Pseudomonas aeruginosa*, *Micrococcus denitrificans* and *Bacillus licheniformis* under both aerobic and anaerobic conditions have shown that the amount of gas produced is equivalent to the amount of nitrate reduced; a similar result was obtained using nitrite instead of nitrate.

Nitrifying bacteria. Preliminary studies have been made of the nutrient requirements of *Nitrosomonas europea* and *Nitrobacter agile* with special reference to trace metals.

Nucleic Acids. Ribose-nucleic acid (RNA), deoxyribose-nucleic acid (DNA) and protein were determined in various fractions of extracts of *Neurospora* felts; the RNA content was about three times that of DNA.

Sulphur Metabolism.

In a study of the growth of *Neurospora* on a number of organic and inorganic sources of sulphur it was found that sulphate, sulphite and thiosulphate gave better growth than the more reduced compounds of sulphur. The reduction of sulphate to cysteine was achieved in extracts of acetone powders of *Neurospora*; the production of cysteine sulphinic acid was accelerated by adding pyridoxal and *dl*-serine.

Biochemistry.

The Effect of Nutrient Status on Metalloporphyrin Compounds in Leaves.

The survey, started in 1957, of the relationship between haem and chlorophyll levels of normal and chlorotic leaves has been extended to a greater range of plant species and of micronutrient deficiency states. In general the close interdependence of the concentrations of the two types of metalloporphyrin has been confirmed. An exception was, however, observed in leaves of *Phaseolus vulgaris* in which acute chlorosis had been induced by withholding manganese. In these leaves, with a chlorophyll content less than 6% of normal, the total haematin content remained high, at 48% to 80% of that in normal leaves, and the characteristic cytochrome components of the chloroplasts could be observed by direct spectroscopic examination of the intact leaf. Chloroplasts isolated from the chlorotic leaves showed a spectrum similar to that of the yellow plastids isolated from leaves of barley germinated in darkness. It now appears that the condition in the manganese-deficient leaf is the result not of a differential effect of the metal on haem and chlorophyll synthesis but of a function of manganese in protecting chlorophyll from the bleaching action of sunlight. This effect is being studied further.

Isolated Chloroplasts.

Studies concerned with the intracellular location of haematin in leaves of a variety of plant species have confirmed that between 50% and 70% of the total cell haematin occurs in the chloroplast as cytochromes b_6 and f . The relative proportions of these characteristic cytochrome components have been measured by differential extraction procedures. The concentration of b_6 was found to be three times that of f ; this measurement takes into account the observation that the extinction in the α -band of reduced cytochrome b_6 is 80% that of cytochrome f .

Observations have also been made on the enzymic activities of protein fractions extracted from isolated chloroplasts, in an attempt to relate them to the dark reactions of photo-reduction and photo-phosphorylation. In this connection a protein fraction isolated from spinach chloroplasts is highly active in catalyzing the reduction of methaemoglobin when reduced triphosphopyridine nucleotide serves as hydrogen donor. In a similar system the protein will also catalyze the reduction of cytochrome b_6 and may therefore be of significance in oxidation-reduction reactions involving the chloroplast cytochromes. Purification of the active material is proceeding.

Organic Chemistry.

The occurrence of a glucoside of 3'-hydroxy-phloretin in certain apple species has already been reported. The glucose residue has now been shown to be at position 4, in contrast to phloridzin itself in which the sugar residue occupies position 2.

The apple species *Malus trilobata* has been found to contain another unusual dihydrochalcone derivative. It is apparently a monoglucoside isomeric with phloridzin, but differing in the position of attachment of the glucose residue: the precise orientation is not yet established.

Small amounts of what appears to be an arabinoglucoside of phloretin have been found in the bark of the cider apple Yarlington Mill. The evidence so far is purely chromatographic, and it is not yet known whether the substance is present in other varieties.

The discovery of these dihydrochalcone glycosides containing a sugar other than glucose, or differing in the point of attachment of the sugar, expands the dihydrochalcone group considerably; the only members previously characterised (phloridzin and asebotin) both carried glucose at position 2.

It has been found that the usefulness of the Craig counter-current extraction apparatus for the separation of complex mixtures of phenolic compounds can be much increased by the successive use of two organic solvents over the aqueous layer, using the less polar solvent first. The method has been used in the examination of the complex mixture of flavonol glycosides found in pear leaf and apple fruit. Isopropyl acetate, as first solvent, extracts quercetin monopentosides; ethyl acetate then removes monohexosides, while diglycosides are left in the aqueous layer.

Compared with the flavonol glycosides of apple leaf reported last year, apple fruit appears to lack the rhamnoglucosides of quercetin and kaempferol. Pear leaf contains both rhamnoglucosides, but xylosides and rhamnosides are absent; also, the amount of kaempferol present is much higher relative to quercetin than in apple samples.

Inorganic Chemistry.

The fluorescent properties of a large number of metal-8-hydroxy-quinoline (oxine) compounds were studied to determine their detection sensitivities. In a series of oxine complexes the sensitivities decreased in the order :



Further work led to the conclusion that fluorescence in oxine complexes occurs when the metal atom in the complex forms cations of only one stable valence state in which the remaining electron shells are in a symmetrically stable state, i.e. empty, half-full, or complete. Oxine complexes of rubidium, caesium, germanium, tin and tantalum, which had not been previously reported, were prepared and analysed. The analyses corresponded to the formulae $\text{RbOH} \cdot \text{C}_9\text{H}_7\text{ON}$, $\text{CsOH} \cdot \text{C}_9\text{H}_7\text{ON}$, $\text{Ge}(\text{OH})_2(\text{C}_9\text{H}_6\text{ON})_2$ and $\text{Sn}(\text{C}_9\text{H}_6\text{ON})_2$; the tantalum compound gave reproducible analyses but could not be assigned any simple formula.

Investigations of metal-morin and metal-riboflavine systems suggested that fluorescence depended on a cationic structure similar to that in the oxine compounds; no definite metallo-compounds of morin or riboflavine could, however, be prepared.

Work was begun on the chromatographic separation and determination of the trace metals normally occurring in soils. A *n*-butanol-HCl-H₂O eluant gave good individual separations of zinc and molybdenum from a mixture containing also iron, cobalt, nickel, copper, manganese and magnesium; the last group was resolved in an acetone-HCl-H₂O eluant. It is hoped to adapt these methods to the examination of soils.

Soil Biology.

In further work on microbiological activity in mineral-deficient soils, it has been found that large variations in activity can be brought about by changes in the carbohydrate supply, or by supplementing the deficient minerals. In the presence of carbohydrate a high level of microbiological activity results in an increase in the proportion of soil aggregated into crumbs, whereas in the absence of adequate carbohydrate, disintegration of crumbs occurs: the more active the microbiological flora the more rapid is the destruction of aggregates.

During the formation of soil aggregates there is a decrease in solubility of some of the mineral nutrients, which again become soluble on the breakdown of the aggregates; work begun with potassium and magnesium has now been extended to copper, manganese and zinc. It is hoped to investigate the effects of these changes in solubility on the growth of higher plants. A method for the assay of trace elements in soil, using paper chromatography as a means of separation (see above) is expected to simplify the determination of trace elements in soil extracts.

THE EFFECTS OF SEED REMOVAL ON THE GROWTH OF APPLE FRUITLETS.

By D. L. ABBOTT.

Introduction.

That fruit development depends upon the presence of seeds is well established, for if fertilization is prevented, fruit set does not occur and the flower abscises. Seeds, particularly the endosperm, have been shown to be a rich source of auxin (1, 2, 3, 5) and this has led to the belief that the mechanism by which seeds control fruit development and growth is hormonal in nature. Further, the parthenocarpic development of certain fruits, e.g. tomato and fig, can be stimulated by the application of auxin to unpollinated flowers.

Attempts to induce such parthenocarpic development of pomaceous fruits, however, have usually failed, but Luckwill (2) has demonstrated a correlation between the levels of auxin in the seed and the successive waves of fruit drop in the apple which suggests that here too the control of fruit abscission is exercised by the seed and that this control is also hormonal.

No attempt has been made in the experiments described below to investigate the immediate post-fertilization stimulus to fruit growth, but only to test, by direct methods, the hypothesis that the continued growth and development of the apple depends upon the presence of seeds. For this purpose open pollination was allowed to take place and, following initial fruit set, seeds were removed or destroyed to determine the effect upon subsequent fruit growth.

Experimental.

The seeds of the apple are totally enclosed by the flesh of the fruit and consequently their destruction inevitably necessitates some damage to the fruit itself. Attempts to destroy the seed by piercing it with a needle have not proved very satisfactory for, though the position of each carpel can be readily determined relative to the sepals, the carpels usually contain two seeds lying one slightly above the other, and both seeds cannot always be punctured by a single prick. A somewhat more reliable seed kill can be obtained by using a hypodermic needle and injecting a small quantity of alcohol into each carpel. This method works well when the seeds are young and delicate, but does not ensure a complete kill in the later stages.

A more drastic but highly effective method is to cut off the distal portion of the fruitlet, thus exposing the seeds, which can then be removed. In a pilot experiment this was done by making a transverse cut around the equator of the fruitlet with a scalpel and lifting off the severed portion. To ensure that this considerable wounding did not materially affect the further growth of the fruitlet, it was necessary to include treatments in which the top was removed but in which the seeds were left intact. Difficulty was experienced in making a sufficiently deep cut through the flesh without causing damage to some of the seeds, and therefore a special tool was devised (Plate I, Fig. 1). Interchangeable stainless steel blades are recessed and bevelled to a sharp cutting edge. By selecting the blade appropriate to the size of the fruit, the flesh can be cut quickly and with comparative ease while minimizing the risk of damage to the seeds.

To prevent water loss the cut surface was immediately sealed with a lanolin emulsion (4) mixed to a smooth creamy consistency. In those treatments in which growth substances were applied, the α -naphthalene acetic acid (NAA) or indolyl acetic acid (IAA), dissolved in alcohol, was mixed into melted lanolin before the preparation of the emulsion.

The following experiments were carried out :—

Experiment I.

Fruitlets of Cox's Orange Pippin were treated as below on 14th–15th June, 1954, i.e. four weeks after petal fall.

Treatment 1—Control.

2—Fruitlets cut to expose seeds, but seeds left intact.

3—Fruitlets cut and seeds removed.

4—Fruitlets cut, seeds removed and 0.5% IAA in lanolin emulsion applied.

5—Fruitlets cut, seeds removed and 0.5% NAA in lanolin emulsion applied.

Approximately twenty adjacent fruitlets on each of five branches of mature bush trees were used for each treatment. The diameters of these fruitlets were measured with calipers weekly until 10th August.

Results. The results of this experiment are shown in Fig. 4.

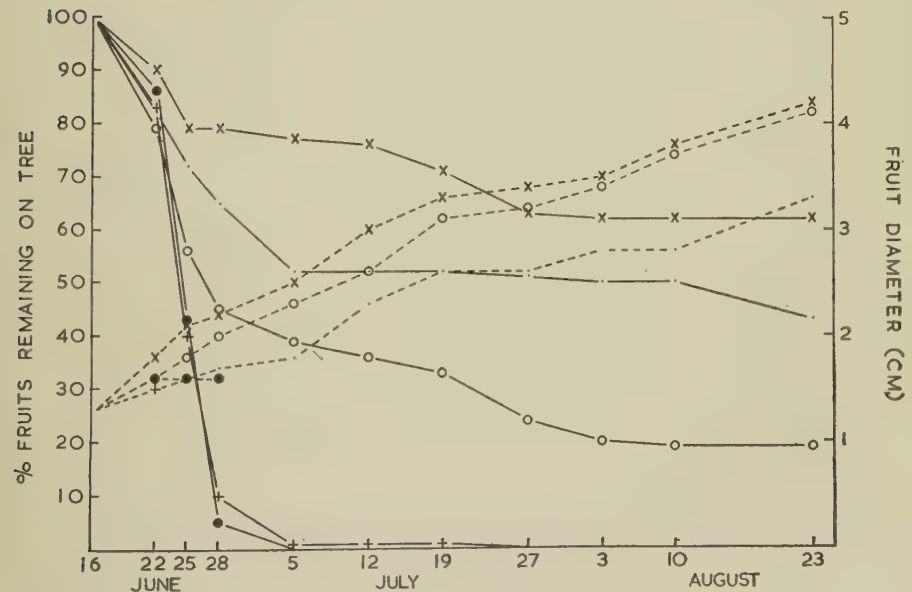


Fig. 4. Effect of treatments on fruit drop and diameter of Cox's Orange Pippin.

x — x Control
 + — + Seeds removed
 — · — Seeds removed + NAA
 o — o Seeds intact
 ● — ● Seeds removed + IAA

Solid line = Number of fruits; Broken line = Fruit diameter.

It is clear that fruitlets can be cut to expose the seeds and yet growth will continue. If, however, the seeds are removed, abscission soon follows, 50% of the fruitlets abscising by the ninth day. The application of 0.5% NAA to the cut surface prevents abscission and the fruitlets continue to grow, albeit at a slower rate than that of the controls. (Plate I, Fig. 2). IAA is without effect and abscission follows treatment with this substance.

Experiment II.

Fruitlets of Crawley Beauty were treated as below at weekly intervals from 14th June–5th September, 1955.

Treatment 1—Control.

2—Fruitlets cut to expose seeds, but seeds left intact.

3—Fruitlets cut and seeds removed.

4—Fruitlets cut, seeds removed and 0.5% NAA in lanolin emulsion applied.

5—Fruitlets cut, seeds intact and 0.5% NAA in lanolin emulsion applied.

Ten adjacent fruitlets, thinned to one per cluster, were used for each treatment. Fruitlet diameters were recorded weekly.

Results. This experiment was designed to determine whether fruitlets respond similarly to treatment at all stages of growth. Table I shows the number of fruits reaching maturity following treatment at weekly intervals after petal-fall.

TABLE I.

NUMBER OF FRUITS (OUT OF 10) REMAINING TO HARVEST FOLLOWING TREATMENT AT VARIOUS STAGES OF GROWTH.

Date of treatment	Weeks after petal-fall	Treatment				
		1 Control	2 Seeds intact	3 Seeds removed	4 Seeds removed + NAA	5 Seeds intact + NAA
14/6	1	7	0	0	3*	3*
20/6	2	10	0	0	1	5
23/6	3	9	3	0	0	0
5/7	4	10	7	0	0	2
11/7	5	8	7	3	3	2
18/7	6	9	6	1	1	5
25/7	7	9	7	6	0	5
2/8	8	9	7	8	0	6
8/8	9	7	10	7	6	5
16/8	10	9	7	5	4	5
22/8	11	8	7	6	5	4
29/8	12	10	9	5	5	6
5/9	13	9	6	6	6	6

*These fruitlets remained firmly attached to the tree, but did not increase in diameter.

These results suggest that the growth of the fruit is dependent upon the presence of seeds only in the early stages of growth, for from about seven weeks after petal-fall, abscission of fruits from which the seeds had been removed (Treatment 3) is not significantly greater than that of fruits with seeds intact

(Treatment 2). Even though the seeds were not removed, abscission followed the removal of the top of the fruit (Treatment 2) in the first two weeks after petal-fall, but this may have been due to faulty technique since the seeds, being young and very delicate, may have been bruised or nicked and so killed during cutting.

NAA is effective in preventing abscission at any time but, if applied one week after petal-fall, it may inhibit further fruit growth at the concentration used. Alternatively, the seeds may at this stage be a source of some growth stimulator and their removal (Treatment 4) or probable death (Treatment 5) would then result in the lack of further growth. The ripening effect of NAA was very marked, and many of the fruits of Treatments 4 and 5 had ripened and become rotten before harvest; the resulting losses account for the somewhat low and erratic results from these treatments. The seeds did not appear to be affected by NAA, even though the lanolin emulsion containing it was smeared over them when the cut fruit surface was sealed. Seeds were collected from fruits of Treatment 5 at harvest, after-ripened and sown on moist cotton wool. Germination was complete and normal; the seedlings were not grown on.

Experiment III.

At weekly intervals from 27th May -1st September, 1956, twenty fruitlets of Cox's Orange Pippin were cut and the seeds removed. Fruit drop was recorded weekly of these fruits and of control fruits on five large limbs of mature bush trees.

Results. That abscission follows seed removal only during the first few weeks after petal-fall suggested a possible association between the time when the fruit becomes independent of the seeds and the ending of the June drop. The purpose of this experiment was to determine whether these two events occur simultaneously. It can be seen from Fig. 5 that the June drop does, in fact, end at that time when seed removal no longer results in fruit abscission.

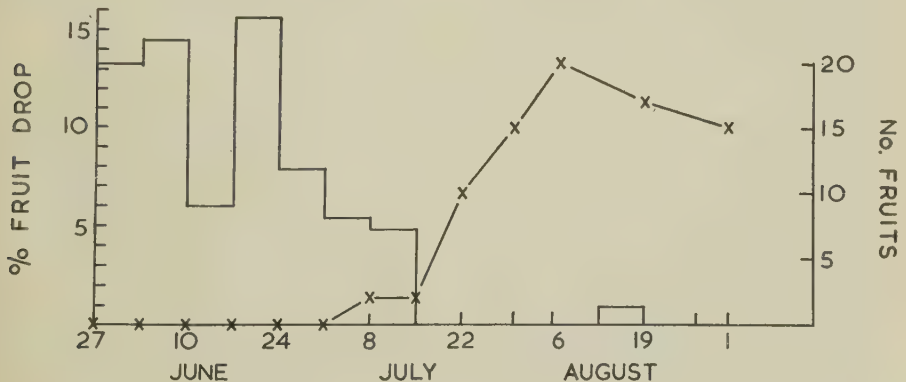


Fig. 5. Fruit drop (histogram) and persistence of fruit from which the seeds have been removed (curve).

Discussion.

The role of the seed during the first one or two weeks after fertilization was not investigated. It appears to be of a complex nature involving the prevention of the formation of an abscission layer, the initiation of thickening

of the pedicel and the stimulation of fruit growth, both by cell division and extension. Such diverse effects very probably involve several different hormonal mechanisms. The results of these experiments, however, suggest that after this initial period, control of fruit growth by the seed is less direct and much more simple, since it can be simulated merely by the application of the single growth substance, NAA. Indeed it seems likely that the only role of the seed, after the initial stimulating stage, is the prevention of fruitlet abscission. Even this control is comparatively short-lived, for a stage is reached when fruit growth proceeds normally in the absence of both seed and added growth substance, and the attainment of this condition, irrespective of any fluctuation in the auxin output of the seeds, may account for the cessation of the June drop. Such a generalization, however, must be confirmed by further work with other apple varieties.

Other investigations, such as the determination of the minimum seed number necessary to prevent fruit fall under various nutritional and competitive conditions, might profitably be carried out by methods similar to those used in these experiments. Although the treatment is drastic, the recuperative ability of the fruitlets is quite considerable, particularly in the early stages, when a meristematic zone develops just beneath the cut surface and gives rise to a corky layer that effectively seals the wound. Plate I, Fig. 3 shows two fruitlets that were injected with a 1% solution of NAA in absolute alcohol. The NAA appears to have been translocated from the fruitlets to the bourse shoots in sufficient quantity to have caused defoliation and death of the shoot tip: yet the fruits, apart from a little blackening of the tissue immediately surrounding the carpels and puncture holes, and a tendency to early ripening, continued to grow normally.

Acknowledgments.

Thanks are due to Mr. E. A. Fribbins for making the cutting tool illustrated in Fig. 1.

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THE MOVEMENT OF PHOTOSYNTHATES IN THE APPLE TREE: I. ¹⁴CO₂ ASSIMILATION TECHNIQUE.

By D. L. ABBOTT and C. P. LLOYD-JONES.

Introduction.

Considerable use has been made of ¹⁴CO₂ in biochemical investigations of photosynthesis and the movement of photosynthates in plants. Most of the published work has been carried out on algal suspensions or on small plants in the laboratory, and has involved the use of elaborate equipment (1). During

PLATE I.

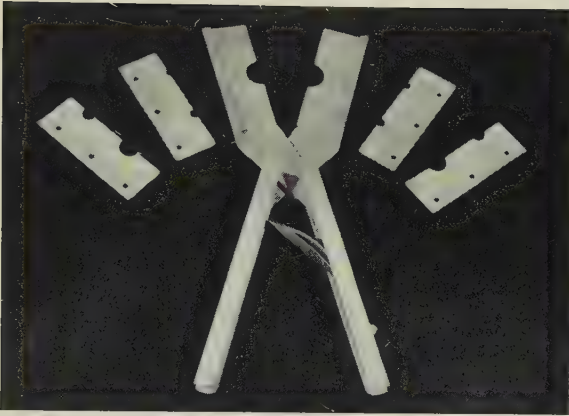


Fig. 1. Cutting tool, showing interchangeable blades.



Fig. 2. Left. Fruits of Cox's Orange Pippin treated with NAA after seed removal on 14th June. Photographed 6th August.



Fig. 3. Right. Fruits of Merton Worcester injected with 1% NAA in absolute alcohol on 29th May. Note defoliation and death of growing point of bourse shoots. Photographed 7th July.

PLATE II.



Fig. 1. Small leaf chamber.

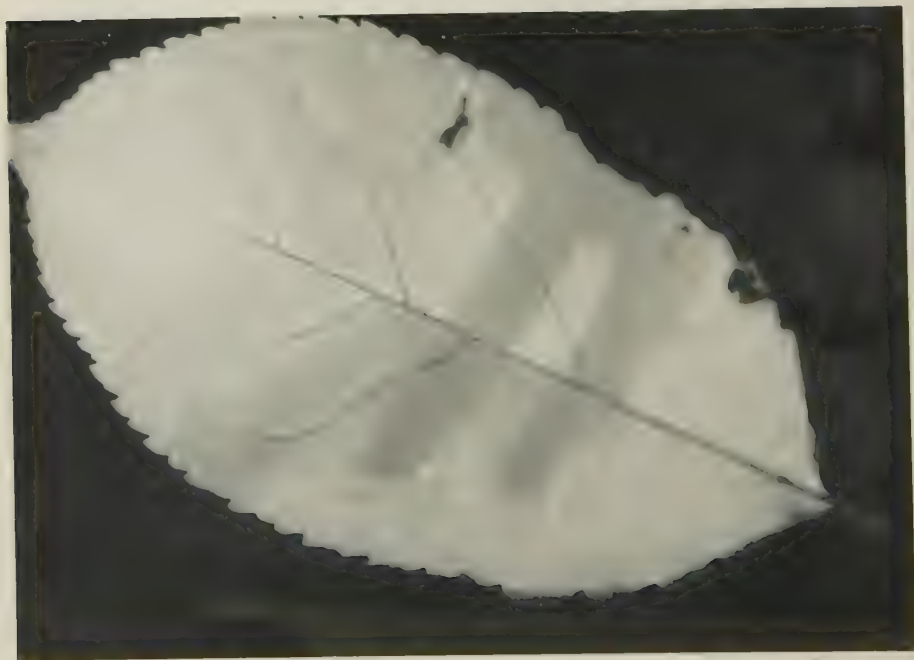


Fig. 2. Autoradiograph of leaf treated with $^{14}\text{CO}_2$ in small leaf chamber.

investigations into the mechanism of the action of α -naphthalene acetic acid (NAA) as a fruit thinning-agent, the need arose for a method of introducing $^{14}\text{CO}_2$ into the leaves of apple trees growing in the glasshouse or orchard. For this purpose a technique using a simple portable apparatus was required. Such an apparatus, consisting only of a Perspex leaf chamber in which a known amount of $^{14}\text{CO}_2$ can be liberated, has been constructed and is described below. While this simple unit proved adequate for the local introduction of $^{14}\text{CO}_2$ into a tree, it was not suitable for comparing the uptake of CO_2 by different leaves. For this purpose a larger chamber capable of accommodating up to 8 detached leaves was designed.

The two types of chamber here described have been used in experiments on the effect of NAA on CO_2 assimilation and distribution which will be reported later.

Small Leaf Chamber.

The small chamber is a rectangular Perspex box, of internal dimensions $10 \times 6.5 \times 1.7$ cm., whose sides are ground to make a gas-tight joint with a Perspex lid. A 2 cm. section of 1.3 cm. internal diameter Perspex tube is joined to the base of the box to form a well, the base of which is sealed by a rubber cap. One end of the box is notched to accommodate the leaf petiole (Plate II, Fig. 1).

Approximately 10 mg. $\text{Ba}^{14}\text{CO}_3$ is placed in the well, and the leaf is laid in the chamber. The petiole is sealed into the notch with modelling clay and the lid is placed in position, the joint being sealed with vaseline; the chamber is then supported and attached to the branch by means of retort stand clamps. 2 ml. ortho-phosphoric acid are injected into the well by inserting a hypodermic needle through the rubber cap sealing the base of the well and allowing the acid to flow on to the $\text{Ba}^{14}\text{CO}_3$; phosphoric acid appears to be the most suitable acid to use since it does not release vapours harmful to the leaves. The leaf is allowed to photosynthesise for one hour before the chamber is removed; by this time most of the $^{14}\text{CO}_2$ has been assimilated, the exact amount taken up not being critical for the present experiments.

Judged by the uniform density of autoradiographs of leaves so treated, adequate dispersion of the $^{14}\text{CO}_2$ occurs in the chamber. It is important to ensure that the whole of the chamber is exposed to light, as shading by adjacent leaves or other obstructions will reduce assimilation, as shown by the shadow of a clamp in the autoradiograph reproduced in Plate II, Fig. 2. The movement of photosynthates from the leaf is followed by sectioning parts of the shoot or branch and preparing autoradiographs.

Large Leaf Chamber.

This apparatus consists of a generator, reservoir and dispensing unit from which a known quantity of $^{14}\text{CO}_2$ can be pumped into an illuminated leaf chamber (Fig. 3). The apparatus is first filled with $^{14}\text{CO}_2$ and calibrated. About 5 g. of $\text{Ba}^{14}\text{CO}_3$ is placed in tube A which, together with bulb B, is then evacuated by a water pump attached to *a*, after filling the left hand limb of the manometer with mercury to tap *c*, which is then closed. Ortho-phosphoric acid is admitted to A from the tap funnel. The $^{14}\text{CO}_2$ generated is sufficient to bring the pressure in the evacuated part of the apparatus to about 74 cm. of mercury. For the calibration of the manometer the leaf chamber E is

replaced by a baryta tube similar to the guard tube D; slight suction is applied to the baryta tube to facilitate the movement of $^{14}\text{CO}_2$ and of CO_2 -free air through the apparatus. Carbon dioxide is drawn from the storage vessel B into the left hand limb of the manometer by lowering the mercury and, after closing tap *c* and bringing the mercury in the two limbs to the same level, the scale is read. Taps *c* and *d* are then opened to the baryta tube; the BaCO_3 precipitated is filtered off and weighed. After correcting the manometer scale reading for temperature and atmospheric pressure a graph is then constructed relating the manometer scale reading to the volume of $^{14}\text{CO}_2$ at N.T.P.

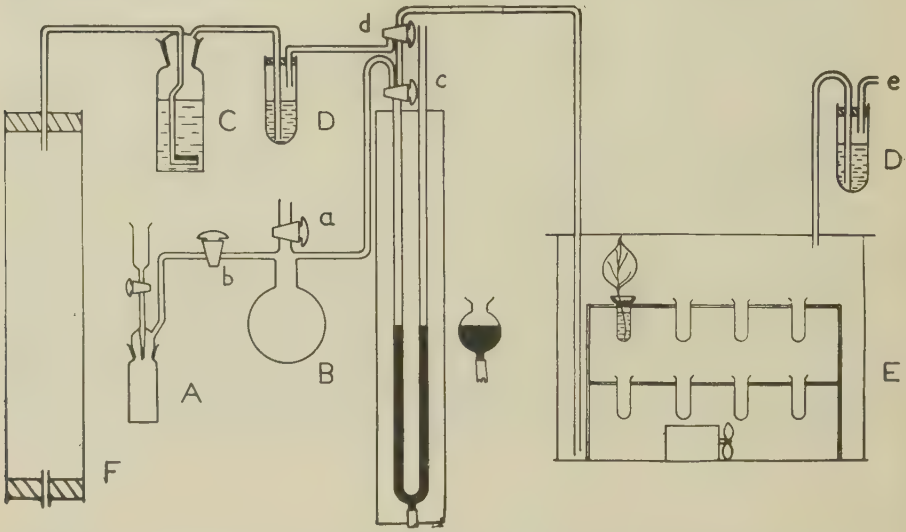


Fig. 3. Large leaf chamber and ancillary apparatus.

After removing the baryta tube the leaf chamber is replaced. Leaves whose CO_2 assimilation is to be compared are placed in small vials with the cut end of the petiole in tap water. The upper end of the petiole is supported by a bung of moist cotton wool and the top of the vial covered by metal foil to minimise solution of the $^{14}\text{CO}_2$ in the water. The rack of leaves is then lowered into the glass chamber E, which is sealed by a vaselined lid. Air is drawn into the chamber through the soda-lime absorber F and the alkali scrubber C by suction at *e*. An aliquot of $^{14}\text{CO}_2$, measured by the manometer, is transferred to the tube connecting tap *d* to the leaf chamber by raising the mercury level, and is then flushed through with CO_2 -free air for 2–3 min. The air stream is then stopped and the fan in the chamber is run to ensure thorough mixing of the $^{14}\text{CO}_2$.

After $\frac{1}{2}$ hr. exposure to 1,400 ft. candles from a 500 watt lamp, the chamber is again swept through with CO_2 -free air and the leaves are removed. They are immediately immersed in boiling alcohol and then autoradiographed or combusted to $^{14}\text{CO}_2$ which is precipitated as $\text{Ba}^{14}\text{CO}_3$, and counted by an end-window Geiger-Muller tube to determine the amount of carbon fixed.

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THE DESTRUCTION OF APPLE TREE STUMPS AND ROOTS BY CHEMICAL MEANS.

By E. W. HOBBS and J. S. COLES.

The fruitgrower has on occasion to thin out trees in overcrowded plantations. The usual method involves complete removal of the roots and the reinstatement of the site to allow the smooth passage of implements. When dealing with large, well established trees, the disturbance of the soil and the roots of surrounding trees can be detrimental; in addition, the grubbing out and disposal of tree stumps can be very expensive. If some satisfactory method could be found of disintegrating tree stumps and roots *in situ*, trees could be felled just below soil surface, leaving the site undisturbed. The use of caustic soda flakes or of oxidizing agents such as sodium chlorate and potassium chlorate has been suggested for forest tree stump destruction and is referred to in a Ministry of Agriculture and Fisheries publication (1).

Few experimental data are, however, available. Curtis (2) described in 1957 work done on apple and pear stumps at New York Agricultural Experimental Station, Geneva, in which stumps of 12-year-old apple and pear seedlings, of 3½-5" diameter at the base, were treated with 80% ammonium sulphamate, with a mixture of 2:4-D and 2:4:5-T, and also with nitrogenous and phosphatic fertilizers. It was found that no treatment produced a rapid decay but that over the 3-year period 1950-1952 the application of nitrogen salts with phosphate (superphosphate) promoted a softening of the stumps.

An opportunity to try chemical means of stump and root disintegration occurred at Long Ashton in 1954 when thinning out trees in a cider orchard of standard trees planted 24 ft. apart in 1903/4. It was decided to compare the effects of some of the substances advocated for stump destruction with those of a fertilizer treatment.

Experimental.

Trees and Materials.

Twenty-four trees, consisting of a number of varieties growing on seedling rootstocks, were sawn off a few inches below turf level. All but two were 51 years old; the average diameter of the 24 exposed stumps was 17". The twenty-four stumps were grouped into six blocks, each of which received the following treatments:

- (i) Aqua regia (1 part conc. nitric acid : 4 parts conc. hydrochloric acid, by volume).
- (ii) Caustic soda (flakes).
- (iii) Bleaching powder.
- (iv) Fertilizer (3 parts Nitro-chalk : 1 part superphosphate, by weight).

Method of Application.

The amount of chemical applied was proportional to the surface area of each individual stump. In December, 1954, holes 6 in. deep and 1 in. diameter were drilled vertically in each stump, using a template, with 1 in. diameter holes at 6 in. square, placed over each stump and adjusted to give the maximum number of holes. The number of holes averaged 7 per stump, the greatest number being 11 and the least 4.

The amount of material to be applied to each hole was calculated from the volume of a hole 4 in. deep (3.15 c. in. = 51.6 c.c.). The quantities used per hole were :

Aqua regia = 51.6 ml.	Caustic soda = 35.0 g.
Bleaching powder = 28.0 g.	Fertilizer = 44.5 g.

The average stump received seven times these quantities.

The acid mixture was poured into the holes through a funnel and the other substances were poured in dry. The holes were left for a few hours and then plugged with corks; the stumps were then covered with a shallow layer of soil and turf.

Records.

Prior to applying the treatments, each stump was assessed for hardness in (a) the heartwood; (b) the perimeter; (c) the roots close to the stump. A scale of 0-5 was used, in which 0 = hard; 5 = soft.

After treatment in December, 1954, the stumps were undisturbed until January, 1957. They were then uncovered and the assessments of hardness repeated; the stumps were then re-turfed. Similar observations were made in February, 1958, and again in late September, 1958, when the orchard area was needed for building operations.

A special look-out was kept for the appearance of the Honey Fungus, *Armillaria mellea*, which was known to be present in the orchard area. No infection by this fungus was noted on any of the treated stumps throughout the period of recording. The fungus *Coprinus* sp. was of common occurrence over some stumps in every treatment; *Mycena* sp. also occurred, particularly over stumps treated with aqua regia and bleaching powder.

Results.

The hardness assessments made in September, 1958, i.e. almost four years after treatment, were subjected to analysis of variance. The heartwood, perimeter and roots were examined separately and allowance was made for the initial scoring made in 1954.

The mean scores per tree are given below, together with the differences needed for significance between two treatments.

<i>Treatment</i>	<i>Heartwood</i>	<i>Perimeter</i>	<i>Roots</i>
Caustic Soda	0.50	1.83	2.17
Bleaching powder	2.50	2.33	2.83
Aqua regia	3.17	3.17	2.83
Fertilizer	3.33	4.00	4.00
<i>Sig. Diff.</i>			
5% level	1.93	1.77	1.80
1% level	2.63	2.41	2.45

Heartwood. Caustic soda was less effective than bleaching powder at the 5% level, and less effective than aqua regia and fertilizer at the 1% level. No other differences between treatments reached significance.

Perimeter. The only difference was that caustic soda was less effective than fertilizer (5% level).

Roots. There was no difference between aqua regia, caustic soda or bleaching powder, but fertilizer was more effective than all other treatments (5% level).

Discussion.

The results of the experiment, although somewhat negative in nature, show that a rapid breakdown of apple tree stumps and roots cannot be expected from any of the materials used in the manner described. Caustic soda flakes have been recommended from time to time, but in this experiment they proved very ineffective; bleaching powder, too, was ineffective. Aqua regia, besides being dangerous to handle, seemed to induce a hardening of the wood immediately around the area of application.

The fertilizer mixture was included in the hope that it would encourage bacterial activity and lead to a more rapid breakdown of woody tissue. The results indicate the usefulness of a nitrogen-phosphate fertilizer in this way and agree with results obtained elsewhere. Fertilizers are simple and safe to apply, are readily available and leave no deleterious effects.

Summary.

A small scale trial is described in which the disintegration of apple tree stumps and roots by aqua regia, caustic soda, bleaching powder and a nitrogen-phosphate fertilizer was investigated. Four years after treatment the fertilizer was the only material that had induced a softening of the wood. Caustic soda, commonly advocated as a softening-agent, was the least effective of the materials tried.

Acknowledgments.

Thanks are due to Mr. R. W. Marsh for his interest throughout the experiment and to Mr. G. M. Clarke for the statistical analysis of data.

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THE EFFECTIVE DISTANCE OF A POLLEN SOURCE IN A CIDER APPLE ORCHARD.

By R. R. WILLIAMS.

Studies of the pollination requirements of cider apples, made during the past six years, have given an indication of the distance over which a pollinator is effective in a mature orchard of standard trees.

No information of this kind appears to have been published for dessert or culinary apples, although it is accepted that in standard orchards the minimum

requirement is one pollinator in nine trees. It is understood, however, that data collected for Bramley's Seedling by Dr. W. S. Rogers at East Malling Research Station between 1920 and 1938, and by Mr. R. W. Kemp, of the National Agricultural Advisory Service, in a Norfolk orchard in 1957, are shortly to be published. The writer is indebted to Dr. Rogers and Mr. Kemp for being allowed to see some of their data, which suggest that the influence of the pollinator extends between 70 and 100 ft.

Experimental.

The records relate to two standard orchards in Herefordshire. The first orchard, a triangular plant, consists of Bulmer's Norman, a triploid variety, with a few well-scattered trees of Michelin, a diploid variety which is an effective pollinator for Bulmer's Norman. The Bulmer's Norman trees have grown into each other, giving a continuous canopy.

In 1956, records of fruit set on the Bulmer's Norman trees around a single pollinator tree of Michelin were taken. Groups of six units, each consisting of a small branch with fifty to one hundred blossom clusters, were disposed radially round the pollinator tree at distances of 10, 40, 50, 80, 90 and 120 ft. Records of the set per 100 blossom clusters five weeks after full blossom are given in Table I; the values obtained three and nine weeks after full blossom were similar. These results indicate that the pollinator tree was effective over a distance of about 50 ft.

TABLE I.
THE EFFECT OF MICHELIN ON THE POLLINATION OF BULMER'S NORMAN, 1956.
Fruit set per 100 blossom clusters 5 weeks after full bloom.

	Distance from periphery of pollinator (feet)					
	10	40	50	80	90	120
	72	50	56	39	17	31
	60	44	27	29	23	18
	84	64	13	33	15	24
	44	82	46	11	28	25
	87	25	32	15	19	18
	31	21	19	15	20	22
Mean	63	48	32	22	20	23

The second orchard contains alternate rows of Bulmer's Norman and Michelin, but a few gaps in the Bulmer's Norman rows have been replanted with Michelin trees. Bulmer's Norman is ineffective as a pollinator, and while Michelin will set fruit without cross pollination, its seed content is then very low. The only pollinators therefore available for the Michelin are a row of Annie Elizabeth trees in an adjacent orchard.

In 1956, 1957 and 1958 seed-counts were made in two samples of 50 Michelin fruits taken at distances of 30 to 510 ft. from the row of Annie Elizabeth (Table II). It will be seen that there is an increased seed content in fruits taken near the Annie Elizabeth. The weather favoured insect activity during the 1956 blossom period but was less favourable in 1957 and 1958; this is reflected in the higher seed content found in fruits taken from the Michelin adjacent to the pollinators in 1956. There is little indication, however, that the conditions in 1956 increased the effective distance of the

pollinator trees compared with 1957 or 1958 : in each year the seed content at 90 ft. is much greater than at 150 ft. 1958 was an unusual season, for unfavourable weather conditions at blossom time were followed by an exceptional set of fruits which had a very low seed number compared with those of the two previous years.

TABLE II.

SEED CONTENT OF MICHELIN APPLES POLLINATED BY ANNIE ELIZABETH.

Means per fruit from two samples of fifty fruits at each distance*

		Distance from pollinator trees.									
Time after full blossom		30'	60'	90'	150'	210'	270'	330'	390'	450'	510'
1956	Weeks :										
	5	5.8	3.1	3.0	1.8	1.6	1.1	1.4	—	—	—
	9	6.3	3.5	3.5	2.6	2.5	1.4	2.0	—	—	—
	19	6.4	4.5	3.4	2.5	2.7	1.9	2.4	—	—	—
1957	5	3.2	3.1	2.8	2.4	2.2	2.3	2.0	2.1	1.9	2.3
	8	3.1	2.9	3.1	1.5	1.3	1.3	0.9	1.1	0.8	1.2
	17	3.4	3.3	3.2	2.1	2.0	1.7	1.2	1.0	1.1	1.4
1958	5	3.1	0.4	0.9	0.5	0.2	0.2	0.6	0.2	0.1	0.3
	7	3.3	1.0	2.0	0.5	0.4	0.2	0.5	0.5	0.3	0.2
	8	3.7	1.2	1.3	0.7	0.2	0.2	0.3	0.4	0.2	0.4
	9	3.8	1.4	1.6	1.0	0.5	0.5	0.5	0.7	0.3	0.3
	13	4.3	1.5	2.0	0.6	0.7	1.0	0.4	0.5	0.6	0.7
	20	4.5	1.5	2.2	0.9	1.3	0.9	0.3	0.7	0.8	0.7

* Only one sample of fifty fruits used at 60'.

The data from these two orchards indicate that the limit of the effective distance of a pollinator apple tree is 50–90 ft. The shorter distances recorded in the first orchard may be due to two factors : (a) the source of pollen was a single tree rather than a row of trees ; (b) the trees formed a continuous canopy of blossom with no inter-tree spaces. Weather conditions at flowering time seem to influence the seed number in fruit adjacent to the pollination trees but do not alter the range over which insect activity is apparent.

Acknowledgment.

I am indebted to Mr. J. H. Hawkins of Thinghill Court, Withington, Hereford, for the facilities given in his orchards.

VIRUS DISEASE IN CIDER ORCHARDS: I.

By A. I. CAMPBELL and R. R. WILLIAMS.

Introduction.

During recent years there has been an increasing interest in the improvement of cider orchards, one result of which has been the extensive headworking of out-dated varieties to more recently recommended ones. The performance of trees treated in this way has not always been satisfactory, and a request for an investigation into the problems involved was made by the Cider Advisory Committee.

Preliminary observations suggested that some of the difficulties encountered were associated with apple mosaic virus. A survey of cider orchards was therefore carried out to determine the amount and distribution of apple mosaic and other viruses. This investigation was confined to Herefordshire and Somerset because of the greater interest in propagation and orcharding in these counties.

Previous work at Long Ashton (1) had indicated that many of the cider varieties are tolerant of apple mosaic, and it was decided that scion nurseries at Long Ashton and elsewhere should be virus-tested to determine whether latent viruses were present.

Orchard Survey.

Eighty cider orchards in Hereford and Somerset were examined during the summers of 1957 and 1958: of these, fifty-four showed visible symptoms of apple mosaic in some of the trees. Symptoms resembling rubbery wood and chat fruit viruses were also seen.

Records suggested that the trees fell into three categories:—

- (a) *Trees planted before 1918.* These were for the most part healthy and only an occasional tree was infected with mosaic.
- (b) *Trees planted from 1918–1946.* During this period mosaic seems to have become more prevalent, particularly in the Herefordshire orchards.
- (c) *Trees planted or headworked from 1946 onwards.* The trees in this group, particularly the recently headworked ones, contained a higher proportion of mosaic infection than the older trees.

Changes in methods of propagation have been major factors contributing to the build-up of mosaic over this period. Orchards planted before 1918 contained a large number of varieties, most of which were of very local distribution and were therefore not subjected to intensive propagation. The variety was usually grafted directly on to seedling rootstocks.

In parts of Somerset, where for fifty years or more it has been customary to use a stembuilder, the traditional variety used for this purpose was Morgan's Sweet. As this variety is very susceptible to mosaic, infected trees were readily seen and eliminated in the nursery; a further opportunity to remove infected stocks occurred in the orchard during the two- or three-year period which elapsed before grafting to the scion variety. Later, with the introduction of Bulmer's Norman as a stembuilder, mosaic became more prevalent, particularly in the Herefordshire orchards where this stembuilder was widely used.

Bulmer's Norman is partially tolerant to mosaic and usually shows no symptoms; hence infection was not readily seen unless the trees were top-grafted with a susceptible variety.

In the immediate post-war period there was a demand for trees to fill up orchard gaps, and many new orchards were planted. This led to a widespread search for scions, particularly of a few high-yielding varieties. Since few young trees were available, the demand was often met by headworking established trees and later distributing the scions from them. In addition, many unpopular culinary, dessert, and cider varieties were headworked to more recently recommended cider varieties. In Somerset and Herefordshire the culinary varieties Bramley's Seedling, Ecklinville Seedling, Newton Wonder, Wellington, and Warner's King have been headworked to cider varieties in large numbers since 1946. The results have been variable: Bramley's Seedling and Newton Wonder have generally given satisfactory results and trees have resumed cropping within a few years; the other three varieties have been unsatisfactory because many of the trees were carrying mosaic, but showed leaf symptoms only when a susceptible cider variety such as Yarlington Mill or Michelin was worked on to them.

The headworking of trees carrying mosaic is usually satisfactory, but often grafts fail, or make only poor growth and sometimes die later. Consequently the tree does not make a good head; crops are poor and the orchard does not show the anticipated improvement in returns.

Infection has also been spread by faulty orchard practice. When headworking is being carried out it is usually impossible to obtain enough scions to graft the whole orchard in one year, even if sufficient skilled labour is available. Hence some of the trees are worked in the first year and these are subsequently used as mother trees to provide scions for further headworking. Though the initial scions may have been healthy, a virus present in the mother tree can be rapidly distributed through the orchard in this way.

It has been the practice for many years to introduce varieties from other cider-growing counties and from France. To obtain a quick assessment of their value these have frequently been headworked on mature trees; this has sometimes led to the infection of the introduced variety and of all the trees subsequently worked with it.

Nursery Survey.

To provide healthy and correctly named cider scions for grafting in the nursery and in the farm orchard, mother trees have been established for many years at Long Ashton and other large nurseries, where they have received special attention to reduce pest and disease attack. Any showing virus symptoms have been destroyed.

Since no information was available on the distribution of latent apple mosaic, rubbery wood and chat fruit in the scion mother trees, this has been examined in the Station nurseries and in one of the larger commercial nurseries. Healthy scions of Lord Lambourne, a variety susceptible to these viruses, were grafted on each tree, the grafted branch being clearly marked with white paint.

The combined results from the two nurseries are shown in Table I.

It will be seen that both apple mosaic and rubbery wood viruses are frequently latent among cider varieties, some of which carry both diseases.

TABLE I.

INCIDENCE OF APPLE MOSAIC AND RUBBERY WOOD IN TWO CIDER SCION NURSERIES.

Variety	No. of trees tested	Healthy	Apple mosaic	Rubbery wood	Suspect rubbery wood	Suspect chat fruit
Ashton Brown Jersey	12	11	—	1	—	—
Bulmer's Norman ..	33	19	1	6	6	1
Court Royal ..	8	5	—	3	—	—
Dabinett ..	17	—	17	2	—	—
Kingston Black ..	22	17	2	2	—	3
Lambrook Pippin ..	5	—	5	—	—	—
Médaille d'Or I ..	12	8	1	—	3	—
Michelin ..	8	6	—	2	—	—
Morgan's Sweet ..	10	10	—	—	—	—
Reine des Pommes ..	6	6	—	—	—	—
Reinette Obry ..	6	—	4	5	—	—
Sweet Alford ..	15	9	5	—	—	1
Sweet Coppin ..	14	10	—	4	—	—
Stoke Red ..	10	8	—	—	—	2
Tardive Forestier ..	10	10	—	—	—	—
Tremlett's Bitter ..	12	11	—	—	—	1
White Norman ..	5	1	4	—	—	—
Yarlington Mill ..	33	14	2	6	9	2
Total ..	238	145	41	31	18	10

Infection by rubbery wood virus may be partly due to the use of vigorous clonal rootstocks prior to 1946. It has been shown (2, 3) that many of these rootstocks carry latent infection. The presence of chat fruit virus has not yet been definitely established, but a number of trees showed symptoms typical of the disease; this may be confirmed at a later date when further crops from the Lord Lambourne scions are examined. The results suggest that some of the trees propagated since 1946 from these scion mother trees will be carrying one virus or more, and that there will also have been infection from headworking with scions from these sources.

In the variety Dabinett all the sources so far tested are infected with apple mosaic; since trees 60 or 70 years old were found to be carrying latent infection, this infection is not likely to be of recent origin. Trees of many varieties have been found free from apple mosaic, rubbery wood and chat fruit. These are being multiplied on seedling apple rootstocks, which are presumed to be virus free, to ensure a supply of healthy propagating shoots from which new scion nurseries can be established.

Other suspected virus diseases have been found in many of the cider varieties; their effect on the vigour and cropping of the trees is being studied.

Summary.

A survey of eighty cider orchards has shown that difficulties encountered in headworking apple trees in West Country farm orchards are in part due to apple virus diseases. Apple mosaic was found to be more prevalent in trees planted or headworked in recent years, particularly since 1946. This is partly attributable to changes in methods of cider apple propagation, including the introduction of stem builders and the replacement of numerous local varieties by a few widely propagated varieties.

The introduction of scion nurseries, in which it was possible to rogue mother trees showing virus symptoms, has helped to minimise the rapidity of the spread in susceptible varieties, but many of these nursery trees have been shown to be carrying latent viruses. Work is now in progress to obtain virus-tested material of all the recommended cider varieties so that new scion nurseries may be established.

Acknowledgments.

The authors are grateful to the many cider growers, manufacturers and N.A.A.S. Officers whose co-operation made this survey possible.

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A SURVEY OF PERRY PEARS IN THE WEST MIDLANDS : II. SOUTH-WEST GLOUCESTERSHIRE.

By GILLIAN NORTH-SMITH.

A survey of the chief perry-growing areas in Gloucestershire, Herefordshire and Worcestershire was begun in 1952. Its objects were outlined in an account of the distribution of perry pear varieties in north-west Gloucestershire (1). Detailed pomological descriptions of some of the more important varieties were given at that time (2) and others have since been published (3, 4).

The survey of south-west Gloucestershire has now been completed (Fig. 1). It has not given such a large number of varietal names as that obtained in the north-western part of the county. A few well known varieties, Arlingham Squash, Barland, Blakeney Red, Butt, Moorcroft, Red Longdon, Oldfield and the Yellow Huffcap made up a large proportion of the trees.

A few varieties that had been named in the first survey were present in south-west Gloucestershire but had no known name. Some new varieties were found, and additional synonyms obtained for previously recorded varieties. Thus the variety Hendre Huffcap, previously recorded only in Worcester (3), was found to be common in Haresfield parish under the name Yellow Huffcap.

As in the first article (1), the distribution shown is that of the name and not that of the variety. The variety Moorcroft, for example, is widely distributed throughout the area under the name of Malvern Hills.



Fig. 1. A Parish Map of South-west Gloucestershire.

Names, Synonyms and Distribution.

The preferred name of each variety is followed by a list of synonyms. Names in square brackets are those given as preferred names in the previous article (1), but not recorded in this area. Varieties which are illustrated in the works of Knight (5), Hogg & Bull (6, 7), Durham (8) or Williams (2, 3, 4) are indicated respectively by the letters K, H & B, D, W2, W3 or W4 placed after the varietal name.

Arlingham Squash	..	W4, H & B, D. <i>Syn.</i> : Squash Pear, Old Squash. <i>Distr.</i> : Many parishes, common in those near the Severn.
Barland	..	K, H & B, D, W2. <i>Distr.</i> : most parishes, common.
Barn Pear	..	W2. <i>Syn.</i> : Brown Thorn Pear. <i>Distr.</i> : Harescombe, Brookthorpe, Eastington, Upton St. Leonards, common.
[Barnet]	..	
Blakeney Red	..	H & B, D, W2. <i>Distr.</i> : all parishes, common.
[Boy Pear]	..	<i>Distr.</i> : Morton Valence. Variety uncommon.
Brown Bess	..	<i>Syn.</i> : Brown Bessie. <i>Distr.</i> : Elmore, Longney, Hardwick, Morton Valence, Fretherne with Saul, uncommon.
Brown Bessie	..	<i>See</i> Brown Bess.
Brown Thorn Pear	..	<i>See</i> Barn Pear. <i>Distr.</i> : Upton St. Leonards, uncommon.
Brown Huffcap	..	<i>See</i> Huffcap. <i>Distr.</i> : Haresfield, common.
Butt	..	<i>Syn.</i> : Norton Butt. H & B, D, W2. <i>Distr.</i> : all parishes, common.
Choke Dog	..	<i>Syn.</i> : Choke Pear. <i>Distr.</i> : many parishes. General term for perry pears.

Choke Pear	See Choke Dog.
Clusters	<i>Distr.</i> : most parishes north of Wheatenhurst, uncommon.
Dandoes	W3. <i>Distr.</i> : Brookthorpe, Haresfield, Hardwick, Morton Valence, uncommon.
[White Longdon]	
Ducksbarn	<i>Syn.</i> : Ducksborne. <i>Distr.</i> : Haresfield, uncommon.
Ducksborne	See Ducksbarn.
[Green Longdon]	W3. <i>Distr.</i> : Haresfield. Variety uncommon, confused with Red Longdon.
Huffcap	W2. <i>Syn.</i> : Brown Huffcap, Uffcap. <i>Distr.</i> : All parishes, common.
[Yellow Huffcap]	See Yellow Huffcap.
Judge Amphlett ..	W4. <i>Distr.</i> : Brookthorpe, uncommon ; distributed by Long Ashton.
Longdon	See Red Longdon. <i>Distr.</i> : all parishes, common.
Mad Cap	H & B, D, W2. <i>Syn.</i> : Mad Pear. <i>Distr.</i> : Fretherne with Saul, Arlingham, Haresfield, uncommon.
[Rock]	
Mad Pear	See Mad Cap. <i>Distr.</i> : Haresfield, uncommon.
Malvern Hills ..	H & B, D, W2. <i>Distr.</i> : All parishes, common.
[Moorcroft]	
Moorcroft	H & B, D, W2. <i>Distr.</i> : Brookthorpe, uncommon ; distributed by Long Ashton.
Norton Butt	See Butt. <i>Distr.</i> : parishes near Severn, common.
Old Squash	See Arlingham Squash. <i>Distr.</i> : Arlingham, Fretherne with Saul, common.
Oldfield	K, H & B, D, W2. <i>Distr.</i> : all parishes, common.
[Pint]	H & B, D, W4. <i>Distr.</i> : Elmore. Variety uncommon.
Red Longdon	K, H & B, D, W2. <i>Syn.</i> : Red Longley, Longdon. <i>Distr.</i> : all parishes, common.
Red Longley (Longney)	See Red Longdon. <i>Distr.</i> : parishes bordering the Severn, common.
Silver Pear	<i>Syn.</i> : Summer Pear. <i>Distr.</i> : Haresfield, Arlingham, Morton Valence, uncommon ; dual purpose pear.
Speart Pear	<i>Distr.</i> : Arlingham, uncommon.
Squash Pear	See Arlingham Squash, Taynton Squash.
Summer Pear	See Silver Pear.
Swan	See Swan Egg.
Swan Egg	<i>Syn.</i> : Swan. <i>Distr.</i> : Eastington, Hardwick and many parishes bordering the Severn, uncommon.
Taynton Squash ..	K, H & B, D, W2. <i>Syn.</i> : Squash Pear. <i>Distr.</i> : Brookthorpe, uncommon ; distributed by Long Ashton. Also another variety with the same name. <i>Distr.</i> : Haresfield, uncommon.
Thorn	H & B, D, W3. <i>Distr.</i> : Fretherne with Saul, Hardwick, Standish, Brookthorpe, uncommon.
[Tumper]	W4. <i>Distr.</i> : Arlingham, Morton Valence, Eastington. Variety uncommon.
Uffcap	See Huffcap, Yellow Huffcap.
Yellow Huffcap ..	W3. <i>Syn.</i> : Huffcap, Uffcap. <i>Distr.</i> : Haresfield, common.
[Hendre Huffcap]	

Acknowledgments.

The author wishes to thank the many farmers and perry-makers without whose help this survey would not have been possible ; also Mr. R. R. Williams for his valuable assistance and advice.

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PERRY PEARS AND THEIR CHARACTERS: III.

By R. R. WILLIAMS and GILLIAN NORTH-SMITH.

In two previous articles (1, 2), twenty-four varieties of perry pear found in the West Midlands were described. The present paper continues the series with descriptions and illustrations of a further thirteen varieties of perry pear from Gloucestershire.

The figures given with each description contain drawings of typical fruits in longitudinal section, together with a flattened spur leaf. The typical winter appearance of mature trees is illustrated by photographs.

Arlingham Squash.

Fruit: 43-56 mm. long, 45-57 mm. diameter. Shape broadly turbinate approaching round. Stalk 10-22 mm.; thick, often lumpy, usually swollen at union with spur. Stem basin usually small and slight, sometimes absent. Eye basin usually slight, sometimes well defined, wide and shallow. Calyx upright, rarely reflexed. Sepals tubular or joined; stamens attached at base of sepals, sometimes red. Skin light green; flush rare, slight, often blotchy, red; russet spreading to cheek; lenticels small, sometimes conspicuous, red on flush; scab slight. Core has axial sac well defined, rarely filled; seeds black. Flesh sometimes tinged yellow, stone cells around core. Taste juicy, sweet, rarely slightly astringent, often pronounced pear flavour and a strong aroma. Harvest 1st-3rd week October. Milling period up to five days after harvesting. Cropping good.

Tree: Small. Crotches wide-angled; long rather slender upright limbs. Thin open branches usually dying back with canker, numerous young shoots.

Flower: Flowering period late mid-season. Flower buds pink. Petals with pink flush, stamens pale red with very profuse pollen.

Leaf: Stalk 23-47 mm. Blade 52-79 mm. long, 32-45 mm. wide; elliptical, inclined to ovate, tip strongly acute or acute, rarely acuminate; base rounded, inclined to cordate; serrations present.

Bartestree Squash.

Fruit: 48-66 mm. long, 47-62 mm. diameter. Shape turbinate, sometimes pyriform. Stalk 23-32 mm.; often connected to fruit by fleshy lip. Stem basin slight, sometimes absent. Eye basin wide and shallow, sometimes well defined. Calyx open, sometimes reflexed. Sepals usually joined, sometimes touching, long, often broken, may be partly green; stamens attached just below sepals. Skin dark green; flush slight to heavy, diffuse; russet around stem and eye, sometimes spreading on to cheek; lenticels usually conspicuous; scab usually present. Core generally has axial sac well defined; seeds dark brown or black. Flesh has stone cells prominent below eye. Taste sweet, usually astringent; possesses a slight aroma. Harvest 1st-2nd week October. Milling-period up to three weeks after harvesting. Cropping good.

Tree: Large. Strong growing; upright, with trunk continued as centre leader. Few limbs. Numerous small very twiggy branches.

Flower: Flowering period mid-season. Flower buds white. Pollen germination very poor.

Leaf: Stalk 19-40 mm. Blade 50-75 mm. long, 37-47 mm. wide; elliptical inclined to ovate, tip acuminate, base rounded; no serrations.

Brandy.

Fruit: 46-58 mm. long, 38-52 mm. diameter. Shape turbinate. Stalk 8-24 mm.; thick, sometimes connected to fruit by a fleshy lip. Stem basin absent. Eye basin slight, wide and shallow. Calyx stiff, upright or slightly reflex. Sepals touching or free; stamens attached at base of sepals. Skin pale green or greenish yellow; flush bright red, usually heavy, sometimes slight; russet more or less covers fruit; lenticels numerous, light brown; scab sometimes present. Core has axial sac, sometimes filled; seeds black. Flesh has a small concentration of stone cells around the eye and a few around the core. Taste juicy, sweet, very slightly astringent; possesses a slight aroma. Harvest 1st-4th week October. Milling period up to 4 weeks after harvesting. Cropping heavy, biennial.

Tree: Small or medium size. Wide-angled crotches. Limbs spreading due to crop. Branches fairly stout.

Flower: Flowering period mid-season.

Leaf: Stalk 16-50 mm. Blade 51-70 mm. long, 33-46 mm. wide; elliptical inclined to ovate, tip acuminate, sometimes acute, base rounded; serrations present, often slight.

Chaceley Green.

Fruit: 36-46 mm. long, 38-53 mm. diameter. Shape oblate or round. Stalk 14-28 mm. Stem basin absent or very shallow. Eye basin shallow or absent. Calyx upright, stiff and prominent. Sepals free; stamens attached at base of sepals. Skin pale green or greenish yellow; flush absent or slight; russet around eye, more around stem, sometimes spreading slightly over cheek; lenticels numerous, usually small, conspicuous; scab absent. Core has axial sac, sometimes well defined, sometimes filled; seeds dark brown. Flesh has small concentration of stone cells below eye, some stone cells around core. Taste juicy, crisp, astringent; possesses an aroma. Harvest 2nd-4th week October. Milling period up to three weeks after harvesting. Cropping heavy.

Tree: Medium size. A few very large limbs spreading due to crop. Small branches with thick growth of twigs.

Flower: Flowering season early. Flower buds white. Petals widely separated, sepals conspicuous. Inflorescence open.

Leaf: Stalk 25-46 mm. Blade 50-63 mm. long, 33-45 mm. wide; elliptical, tip variable, usually acuminate, base rounded, serrations present.

Claret.

Fruit: 25-35 mm. long, 29-45 mm. diameter. Shape oblate or round. Stalk 16-34 mm.; thin, rarely slight swelling adjacent to fruit. Stem basin variable, very slight to well defined. Eye basin often well defined, sometimes slight. Calyx usually strongly reflexed, sometimes open. Sepals tubular or joined, prominent; stamens usually attached at base of sepals. Skin yellow or greenish yellow; flush very rare; russet around stem and eye, spreading over cheek, sometimes virtually complete; lenticels not conspicuous except on russet around stem; trace of scab often present. Core has axial sac, usually

well defined ; seeds black. Flesh has some stone cells around core. Taste juicy, astringent ; pear flavour usually pronounced and a strong aroma when ripe. Harvest 4th week October onwards. Milling period 3 weeks or over. Cropping heavy.

Tree : Large. Wide-angled crotches. Numerous large limbs spreading due to crop. Numerous branches of rather thick growth.

Flower : Flowering period mid-season. Flower buds white or slight flush.

Leaf : Stalk 31-55 mm. Blade 47-63 mm. long, 31-45 mm. wide ; elliptical, tip acuminate, sometimes acute or obtuse, base rounded inclined to cordate ; no serrations.

Coppy.

Fruit : 37-47 mm. long, 34-48 mm. diameter. Shape elliptical or round, rarely oblate or slightly pyriform. Stalk 15-31 mm., sometimes slightly swollen at union with spur. Stem basin absent, sometimes trace. Eye basin absent, occasionally has raised eye, sometimes with slight shoulders. Calyx upright or slightly reflexed. Sepals free, sometimes touching, rarely joined at base ; stamens attached at base of sepals. Skin yellow or greenish yellow ; flush absent or slight, orange, spreading from stem ; russet around stem and eye ; lenticels numerous, usually light in colour ; scab usual, rarely severe. Core has axial sac well defined ; when fruit is ripe sac fills with dark treacly liquid ; seeds black. Flesh tinged green below skin, has slight ring of stone cells around core with small concentration below eye. Taste rather dry, sweet ; moderate astringency, good pear flavour ; possesses an aroma. Harvest 2nd-4th week of October. Milling period up to 7 days after harvesting. Cropping variable.

Tree : Large. Trunk usually continued as centre leader. Acute-angled crotches with a small number of upright limbs. Numerous branches with dense, very thin, twiggy growth.

Flower : Flowering period mid-season. Flower buds white, Petals separate, sepals reflexed and inconspicuous. Rather pale anthers.

Leaf : Stalk 38-59 mm. Blade 56-76 mm. long, 37-51 mm. wide ; elliptical, tip acuminate, sometimes acute ; base rounded inclined to tapering ; serrations pronounced.

High Pear.

Fruit : 32-46 mm. long, 30-46 mm. diameter. Shape round or oblate, often approaching turbinate. Stalk 15-34 mm., usually green, sometimes connected to fruit by a fleshy lip. Stem basin absent, sometimes merging. Eye basin slight or well defined. Calyx open or strongly reflexed. Sepals joined at base ; stamens attached below base of sepals. Skin dark green or green ; flush rare, slight, yellowish red ; russet around eye, sometimes around stem, rarely spreading ; lenticels small brown but conspicuous ; scab absent or trace. Core has axial sac usually open to some of carpels ; seeds black. Flesh has prominent stone cells in thick ring. Taste soft, juicy and sweet, some aroma. Harvest 1-3rd week October. Milling period up to 1 week after harvesting. Cropping heavy but biennial.

Tree : Medium size. Sturdy limb system with characteristic drooping of limbs and branches due to crop. Small branches and twigs often pendulous.

Flower : Flowering period mid-season. Flower buds white. Petals small, making sepals conspicuous ; stamens dark red, styles twisted and considerably longer than stamens.

Leaf : Stalk 29–38 mm. Blade 44–51 mm. long, 37–43 mm. wide ; elliptical inclined to ovate, tip obtuse, rarely acuminate, base cordate, serrations pronounced, sometimes strongly pronounced.

Horse Pear.

SYN. GREEN HORSE.

Fruit : 36–61 mm. long, 45–60 mm. diameter. Shape usually oblate, sometimes slightly turbinate. Stalk 14–44 mm. Stem basin usually small, narrow, fairly deep, sometimes absent. Eye basin often well defined. Calyx usually upright. Sepals joined, sometimes free, sometimes fleshy at base, often pubescent ; stamens attached at base of sepals. Skin green or yellowish green ; flush absent or slight, orange ; russet around stem, more around eye spreading to cheek ; lenticels usually almost white, often conspicuous on russet ; scab often present. Core generally has axial sac, sometimes well defined, sometimes filled ; seeds black. Flesh has ring of stone cells around core. Taste juicy, sweet, with little or no astringency ; sometimes a pronounced flavour ; possesses an aroma. Harvest 2nd–4th week October. Milling period up to three weeks. Cropping fairly good, regular.

Tree : Large. Narrow-angled crotches. Numerous large heavy upright limbs terminating in small stiff branches. A tree of characteristic stiff appearance.

Flower : Flowering period early mid-season.

Leaf : Stalk 25–50 mm. Blade 43–60 mm. long, 35–54 mm. wide ; elliptical, tip obtuse, rarely acuminate, base cordate, occasionally rounded inclined to cordate ; serrations strongly pronounced.

Judge Amphlett.

Fruit : 45–59 mm. long, 36–50 mm. diameter. Shape pyriform. Stalk 10–21 mm. Stem basin absent or very slight. Eye basin absent (rarely slight, wide and shallow) or eye raised. Calyx open, usually stiffly upright. Sepals touching, sometimes free or joined at base, often broken off ; stamens attached at base of sepals. Skin yellow or greenish yellow ; flush rare, may be present on cheek or russet ; russet around stem, more around eye, spreading over cheek ; lenticels usually inconspicuous and small ; scab absent. Core may have axial sac, small, often ill-defined or filled ; seeds dark brown. Flesh has concentration of stone cells below eye and a few around core. Taste juicy, slight to moderate astringency ; flavour often similar to peach ; possesses an aroma. Harvest 3rd week September–1st week October. Milling period up to one week. Cropping regular and heavy.

Tree : Medium size. Trunk usually continued as centre leader. Few limbs with narrow crotches. Numerous branches of dense twiggy growth.

Flower : Flowering season early. Flower buds white.

Leaf : Stalk 30–42 mm. Blade 46–63 mm. long, 32–49 mm. wide ; elliptical, tip obtuse, base rounded ; no serrations.

Lumber.

SYN. LUMBER REDS, STEELYARD BALLS.

Fruit : 60–100 mm. long, 43–65 mm. diameter. Shape pyriform. Stalk 16–28 mm. Stem basin absent or very slight. Eye basin absent or very slight. Calyx open. Sepals joined, sometimes touching, rarely free, long and prominent except when broken ; stamens attached at base of sepals. Skin pale green ; flush heavy, diffuse ; russet spreading, rarely around stem and eye ; lenticels conspicuous except on flush ; scab absent. Core has axial sac well defined ; seeds brown, many abortive. Flesh hard, has stone cells around core, often has greenish tinge. Taste mildly acidic, astringent ; no appreciable flavour or aroma. Harvesting 4th week October 1st week November. Milling period up to eight weeks after harvesting. Cropping heavy, sometimes biennial.

Tree : Small. Sparse limb system with numerous branches, often pendulous.

Flower : Flowering season early. Flower buds white. Blossom large.

Leaf : Stalk 33–50 mm. Blade 55–76 mm. long, 38–49 mm. wide ; obovate, tip acuminate, base tapering ; serrations always present.

Pint.

Fruit : 53–73 mm. long, 43–58 mm. diameter. Shape pyriform, occasionally approaching turbinate or elliptical. Stalk 15–32 mm. ; often connected to fruit by fleshy lip. Stem basin absent. Eye basin absent or slight. Calyx upright. Sepals free, sometimes touching ; stamens attached at base of sepals. Skin yellowish green ; flush absent ; russet around stem and eye, spreading over cheek ; lenticels small and inconspicuous ; scab absent. Core has axial sac well defined ; seeds black. Flesh has a few stone cells around core. Taste juicy, acid, sometimes astringent ; possesses an aroma. Harvest 2nd–4th week October. Milling period up to three weeks after harvesting. Cropping very heavy.

Tree : Large. Narrow-angled crotches, numerous sturdy upright limbs. Numerous branches with twiggy, often pendulous, growth.

Flower : Flowering period mid-season. Inflorescence densely pubescent.

Leaf : Stalk 30–59 mm. Blade 46–70 mm. long, 32–48 mm. wide ; elliptical, tip acute, sometimes acuminate or obtuse, base rounded, sometimes inclined to cordate. Serrations present, often slight. Wavy margin to leaf blade. Undersurface pubescent.

Thurston's Red.

Fruit : 35–44 mm. long, 32–42 mm. diameter. Shape turbinate or pyriform. Stalk 15–35 mm. ; rather thin, often patches of green. Stem basin absent. Eye basin wide and usually well defined. Calyx upright. Sepals touching, sometimes joined, often fleshy at base ; stamens attached at base of sepals. Skin greenish yellow or yellowish ; flush slight to heavy, red ; russet around stem and eye often spreading on to cheek ; lenticels variable, usually inconspicuous ; scab usual. Core has well defined axial sac, rarely open to eye ; seeds black. Flesh has ring of stone cells around core. Taste juicy, sometimes sweet, astringent ; possesses a pronounced pear flavour and strong aroma. Harvest 2nd–3rd week October. Milling period up to three weeks after harvesting. Cropping regular, light to medium.

Tree: Medium. Crotches wide-angled. Trunk continued as centre leader. Few limbs. Numerous small branches forming dense head.

Flower: Flowering period mid-season. Flower buds white.

Leaf: Stem 24–36 mm. Blade 40–59 mm. long, 39–55 mm. wide; elliptical, cupped, tip obtuse, base rounded inclined to tapering; serrations present.

Tumper.

Fruit: 30–43 mm. long, 32–44 mm. diameter. Shape round, slightly oblate, rarely turbinate. Stalk 12–25 mm.; sometimes rather stout, sometimes connected to fruit by fleshy lip. Stem basin usually absent; if present small and narrow. Eye basin slight, wide and shallow. Calyx usually reflexed, sometimes upright. Sepals usually joined, sometimes free, often light coloured; stamens attached at base of sepals. Skin greenish yellow or yellowish; flush slight or absent, red; russet around eye and stem; lenticels very small, brown; scab moderate, sometimes severe. Core has well defined axial sac, seeds black. Flesh has weak or incomplete ring of stone cells around core. Taste juicy, sweet, slightly astringent; possesses an aroma. Harvest 3rd 4th week September. Milling period up to five days after harvesting. Cropping regular but light.

Tree: Large. Numerous limbs with wide-angled crotches, giving a large wide framework. Branches numerous, heavy spur systems.

Flower: Flowering period mid-season. Flower buds white.

Leaf: Stalk 40–60 mm. Blade 50–72 mm. long, 33–45 mm. wide; elliptical, inclined to ovate, tip acute, sometimes strongly acute, base rounded; serrations strongly pronounced.

REFERENCES.

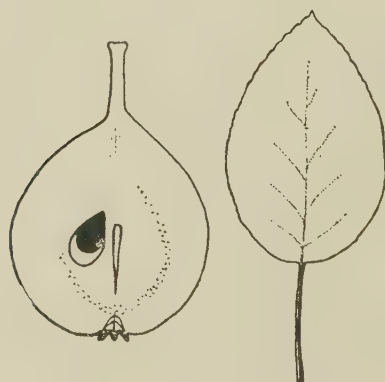
- (1) WILLIAMS, R. R. (1954). Perry pears and their characters: I. *Ann. Rep. Long Ashton Res. Sta. for 1953*, 70–78.
- (2) WILLIAMS, R. R. (1955). Perry pears and their characters: II. *Ann. Rep. Long Ashton Res. Sta. for 1954*, 52–59.

PLATE III.



ARLINGHAM SQUASH.

BARTESTREE SQUASH.



BRANDY.

Fig. 1.

PLATE IV.



ARLINGHAM SQUASH.



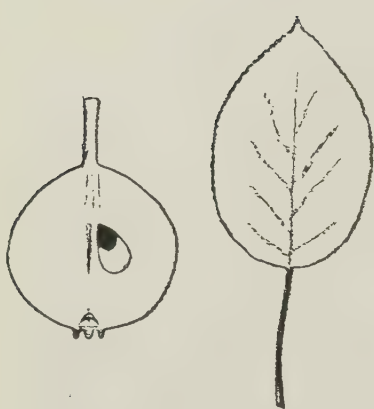
BARTESTREE SQUASH.



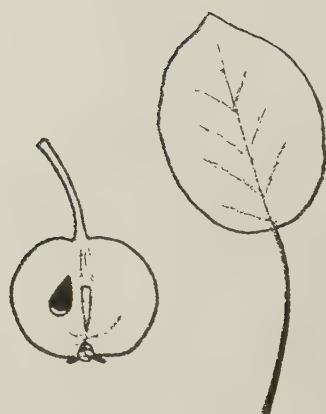
BRANDY.

Fig. 2.

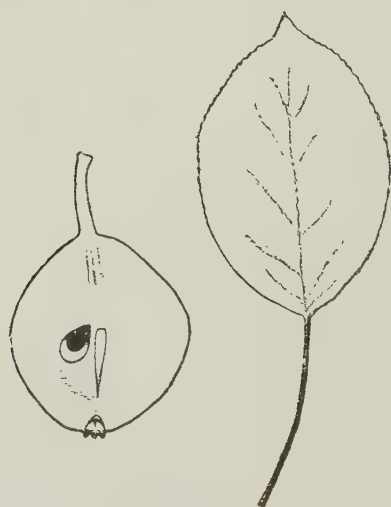
PLATE V.



CHACELEY GREEN.



CLARET.



COPPY.

Fig. 3.

PLATE VI.



CHACELEY GREEN.

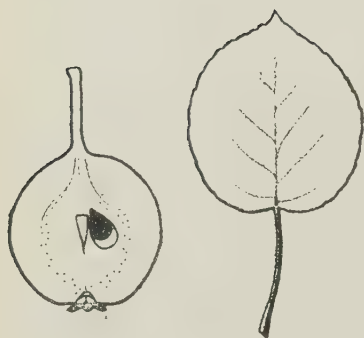


CLARET.

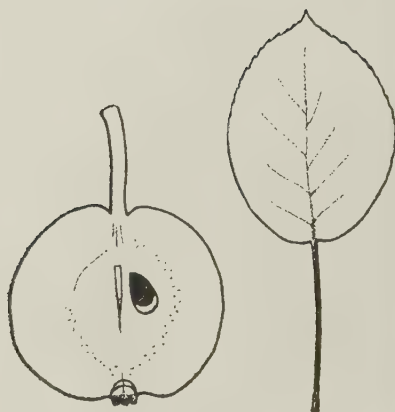


COPPY.
Fig. 4.

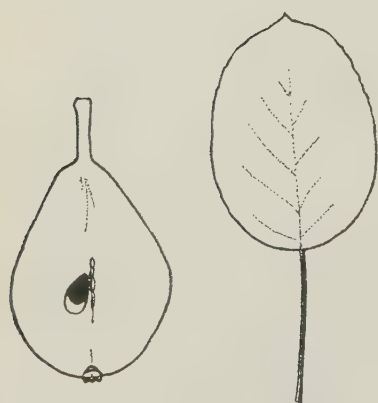
PLATE VII.



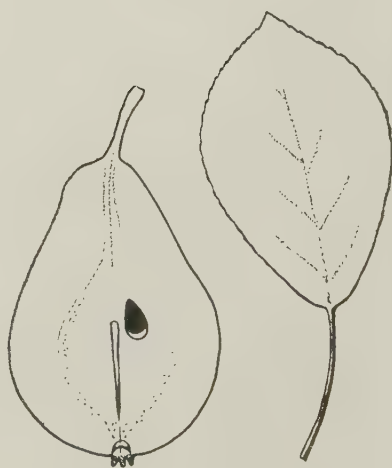
HIGH PEAR.



HORSE PEAR.



JUDGE AMPHLETT.



LUMBER.

Fig. 5.

PLATE VIII.



HIGH PEAR.



HORSE PEAR.



JUDGE AMPHLETT.



LUMBER.

Fig. 6.

PLATE IX.



PINT.

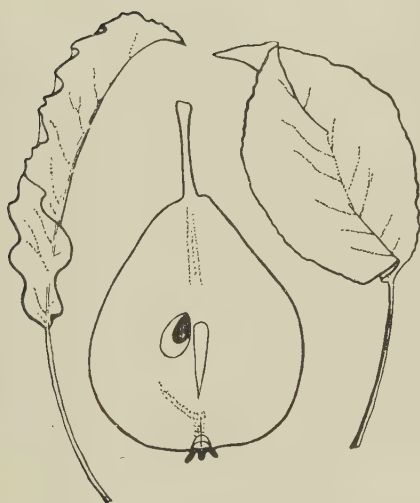


THURSTON'S RED.

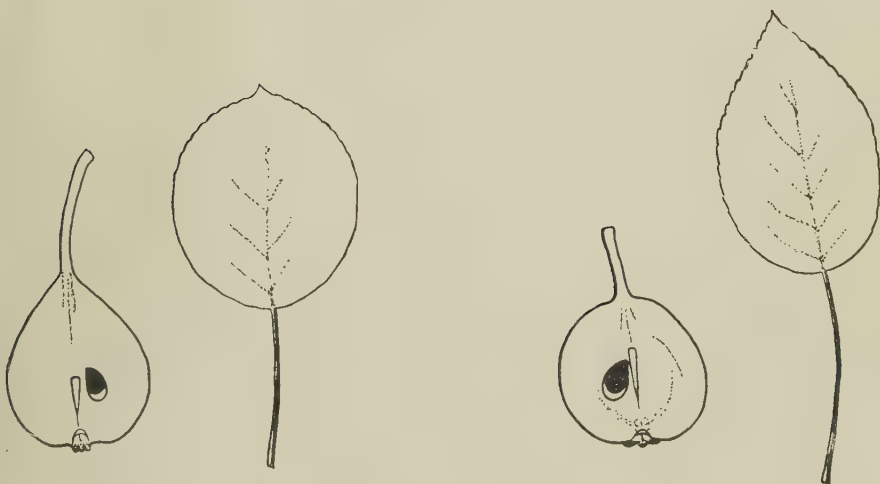


TUMPER.

PLATE X.



PINT.



THURSTON'S RED.

TUMPER.

Fig. 8.

PREDICTION OF HARVEST DATE FOR BLACK CURRANTS.

By D. WILSON.

Introduction.

The date on which a black currant variety can be harvested varies considerably from year to year, and it would be of obvious value to growers and processors if the date could be predicted with reasonable accuracy. Haskell (1) has shown that, in sweet corn, flowering time is a good indication of when the crop will be ready for harvest ; he has also reported a correlation between flowering and harvest dates in the blackberry variety Merton Early (2). The following investigation was undertaken to determine whether flowering date could be used as a basis for the prediction of harvest date in black currant varieties.

Material and Methods.

The data were obtained from black currant plantations at Long Ashton where records of dates on which the first flower opened and of harvest have been collected annually for many varieties. The most extensive records were available during the periods 1946-49 and 1952-58 for the varieties Seabrook's Black, Mendip Cross, Baldwin, Westwick Choice, Malvern Cross, Cotswold Cross and Boskoop Giant, and only these were considered.

Results.

Mean flowering dates for the above varieties are given in Table I, together with the number of days by which the actual date on which the first flower opened deviated from the mean over periods of up to 11 years. Inspection of the mean flowering dates shows that the order of flowering bears no relation to the accepted order of harvest for these varieties. This would seem to indicate varietal differences in the time needed for development from flower to ripe berry. The deviations from mean flowering date for any one year are, with few exceptions, of the same order, which suggests that all varieties react in a similar manner to given climatic conditions.

Table II shows the mean harvest dates for the varieties, together with the number of days deviation from the mean. Unfortunately, the only records available were those of actual harvest date, which does not necessarily coincide with the date on which the fruit was fit for harvest. For example, in 1957 the varieties Boskoop Giant and Mendip Cross were harvested at the appropriate time, but rain then delayed the picking of the later varieties although they were fit for harvest.

The correlation coefficient between the deviations of flowering times and harvest dates from their respective means was found to be $+0.677$ for all years and all varieties. This coefficient is significantly different from zero at the 0.1% level of probability and suggests a definite relationship between date of first flower and harvest date. If the data for 1946 and 1957 (years in which actual harvest date did not coincide with the date on which the fruit was fit for harvest because rain prevented picking) are omitted, the correlation coefficient is increased to $+0.851$, a very satisfactory figure for biological material.

TABLE I.

ANNUAL DEVIATIONS IN DAYS FROM THE MEAN FLOWERING DATE FOR SEVEN BLACK CURRANT VARIETIES.

	Boskoop Giant	Mendip Cross	Seabrook's Black	Cotswold Cross	Malvern Cross	Baldwin	Westwick Choice
Mean Flowering Date	15/4	14/4	19/4	11/4	11/4	11/4	14/4
1946	-12	-8	-11	-5	-9	-9	-8
1947	+9	+10	+6	—	+13	+10	+11
1948	-1	-5	-2	—	-2	-6	-2
1949	-4	-5	-6	-5	-5	-5	-6
1952	—	0	—	+4	+1	+2	+2
1953	—	-4	-4	-1	-4	-4	-3
1954	—	0	-2	+2	+2	-1	-2
1955	—	+10	+8	—	—	+10	+11
1956	+14	+9	+11	+12	+10	+10	+9
1957	-16	-20	-14	-16	-15	-15	-18
1958	+13	+11	+10	+12	+9	+12	+11

TABLE II.

ANNUAL DEVIATIONS IN DAYS FROM THE MEAN HARVEST DATE FOR SEVEN BLACK CURRANT VARIETIES.

	Boskoop Giant	Mendip Cross	Seabrook's Black	Cotswold Cross	Malvern Cross	Baldwin	Westwick Choice
Mean Harvest Date	7/7	8/7	13/7	15/7	19/7	19/7	21/7
1946	+1	0	-5	-4	+4	-4	+3
1947	+10	+8	+4	—	+6	+8	+8
1948	-6	-1	-10	—	-6	-6	-8
1949	-7	-8	-8	-1	-6	-6	-7
1952	—	-6	—	-6	-4	-11	-7
1953	—	-1	-5	-1	-1	-3	-6
1954	—	0	-1	-1	0	-2	0
1955	—	+11	+8	—	—	+7	+6
1956	+4	+6	+4	+3	+7	+8	+6
1957	-10	-11	+2	+2	-2	-2	-2
1958	+8	+7	+9	+8	+6	+7	+8

The number of days between first flower and harvest date was then calculated for each variety in each year; data for the years 1946 and 1957 were again omitted for the reasons given above. Table III presents the mean number of days from first flower to harvest, together with the annual deviations in days for each variety. From the means it can be seen that the varieties fall into two broad groups: Boskoop Giant, Mendip Cross and Seabrook's Black appear to require up to 13 days less than the remaining varieties for development to maturity. It can be seen from Table III that, with few exceptions, predictions based on the mean number of days from first flower would have been reasonably accurate in forecasting harvest date. Of a total of 54 forecasts 67% would have been accurate to within ± 3 days, 30% within 4-6 days, and only 3% would have been wrong by 7 or more days.

TABLE III.
ANNUAL DEVIATION IN DAYS FROM THE MEAN NUMBER OF DAYS BETWEEN
FIRST FLOWER AND HARVEST DATE.

	Boskoop Giant	Mendip Cross	Seabrook's Black	Cotswold Cross	Malvern Cross	Baldwin	Westwick Choice
Mean	79	84	82	91	96	96	95
1947	+5	-1	+1	—	-4	+1	0
1948	-1	+5	-5	—	-1	+3	-3
1949	+1	-2	+1	+8	+2	+2	+2
1952	—	-5	—	-6	-2	-10	-6
1953	—	+4	+2	+4	+6	+4	0
1954	—	+1	+4	+1	+1	+2	+5
1955	—	+2	+3	—	—	0	-2
1956	-6	-2	-4	-5	0	+1	0
1958	-1	-3	+2	0	0	-2	0

Discussion.

The high correlation between flowering time, taken as the date on which the first flowers open, and date of harvest, suggests that the former may be used to predict date of harvest with reasonable accuracy. Varying climatic conditions make absolute accuracy impossible, but the method given should be reasonably reliable for most varieties in most years.

It must be emphasized that, while correlation between flowering date and harvest date will probably obtain in other parts of the country, the actual number of days between flowering and harvest for each variety may differ from that given in Table III. Therefore it is in no way suggested that the means given in Table III should be used for prediction of harvest dates in other parts of the country: rather, flowering date and harvest date records should be collected in the areas concerned.

Summary.

A high correlation between date of first flower and harvest date for seven black currant varieties over periods of up to eleven years suggests that flowering date can be used as a basis for the prediction of harvest date. It is shown that forecasts based on the mean number of days between flowering and harvest date would have been accurate to within ± 3 days in 36 instances out of 54.

While the method will probably be applicable in other areas, environmental differences will make it necessary to base calculations on local data.

Acknowledgments.

Thanks are due to Mr. J. B. Duggan who suggested the problem, to Mr. G. M. Clarke for help in the statistical analysis and to Mr. E. Catlow and Mr. G. E. Clothier for the provision of flowering and harvest data.

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MANURIAL EXPERIMENTS WITH FRUIT: II. A FACTORIAL NPK EXPERIMENT WITH STRAWBERRY, VAR. ROYAL SOVEREIGN.

By C. BOULD.

Previous papers (1, 2) have dealt with the effect of long-term fertilizer and F.Y.M. treatments on soil fertility, crop yield and leaf nutrient status. The present paper deals with the results of the first phase of a more complex field experiment carried out at the N.A.A.S. Horticultural Experiment Station, Efford. It is one of a number of factorial experiments designed to study: (a) the response of fruit crops to manurial treatments under different soil and climatic conditions; (b) the effect of treatments on leaf nutrient status at different physiological stages of growth, and (c) the relationship between leaf nutrient status, growth and crop yield.

Other nutritional experiments with strawberry are being conducted in the field under cloches, and in sand culture.

Experimental.

Site and Soil.

The site is fairly level and the soil is a silty loam (brick earth type), rather low in organic matter and originally very acid. Magnesian limestone was applied at 2 tons per acre in the year prior to planting with strawberries. During this year a light cover crop of Italian rye was grown and ploughed-in.

Chemical data for the soil, prior to liming, are given in Table I.

Layout and Manurial Treatments.

The total experimental area is approximately 0.98 acre and is divided into 3 blocks, each containing 27 plots. A plot consists of 6 rows (only 4 of which are recorded), each row containing 15 plants, plus a row on each boundary between adjacent plots on the E. and W. sides. The N. and S. plot boundaries are not buffered, but a distance of 4 ft. separates the last plants in one plot from the first plants in the next.

Runners of the variety Royal Sovereign were planted in September, 1954, at 18 in. between plants and 3 ft. between rows.

There are 27 treatments, in triplicate, comprising all combinations of three levels of N, P and K. The rates per acre are:—

$N_1 = 1$	cwt. ammonium sulphate
$N_2 = 2$	„ „ „
$N_3 = 4$	„ „ „
$P_0 = 0$	superphosphate (18% P_2O_5)
$P_1 = 2$	„ „ „
$P_2 = 4$	„ „ „
$K_0 = 0$	potassium sulphate
$K_1 = 1$	„ „ „
$K_2 = 2$	„ „ „

Treatments are applied annually in March.

Management.

The first autumn and winter after planting were very wet ; the surface soil was heavily panned, and many plants were buried with fine sandy silt. Some subsequently died ; these were replaced in April, 1955. Plants showing virus symptoms, or suffering from severe wireworm damage, were removed and replaced with healthy plants in September, 1955. A final replacement of missing plants was made in April, 1956. Thereafter a record was kept of weak and missing plants for which a co-variance correction was made in the yield data.

Normal commercial management was practised so far as it was consistent with the object of the experiment. Routine pest and disease control included applications of schradan for aphids, captan for *Botrytis* and Karathane for mildew.

No crop or leaf samples were taken in 1955.

Leaf Samples.

Leaf samples were taken in 1956, 1957 and 1958 from each plot. A plot sample consisted of about 30 leaves, one fully expanded leaf, with the minimum amount of petiole attached, being taken from every other plant in the four recorded rows. Each plot was sampled at flowering and again at fruit ripening. The samples were dried the same day at approximately 100°C., crushed, sub-sampled, and later analysed for total nitrogen, phosphorus and potassium.

Experimental Records.

Data have been collected for plant vigour, plant losses, incidence of disease and deficiency symptoms, yield and grade of fruit, and leaf nutrient status.

Results.

Soil Analysis.

Analytical data for soil samples, taken prior to the addition of limestone, are given in Table I. It will be noticed that the original pH was very low ; this would partly account for the relatively low available phosphate. Exchangeable potassium was medium, and organic carbon (a measure of humified organic matter) was rather low. The pre-planting cover crop of Italian rye grass made poor growth and probably had little, if any, effect on soil organic matter. The addition of limestone at 2 tons per acre had raised the pH to 5.7 by 1954, and this had probably increased the availability of soil phosphorus.

TABLE I.
SOIL DATA PRIOR TO MANURIAL TREATMENTS AND LIMING (1953).
(As % air-dry soil : 0-8 in. depth).

Block	*pH Soil : water ; 1 : 2.5	Organic carbon	P ₂ O ₅ 1 % citric sol.	P ₂ O ₅ N/2 acetic sol.	K Exchangeable
I	4.3	1.35	0.010	0.0024	0.022
II	4.4	1.35	0.008	0.0021	0.018
III	4.1	1.33	0.011	0.0025	0.017

*After 2 tons/acre of limestone the mean pH value in 1954 was 5.7.

Plant Vigour and Symptoms.

After a poor start in 1954, the plants recovered in 1955 and continued to make vigorous growth for the next three seasons. The only visible effect on growth that could be observed was due to nitrogen, N_3 plants being slightly more vigorous and darker green, and N_1 plants less vigorous and paler green than N_2 plants. There was no visible sign of phosphorus or potassium deficiency.

Yield and Grade of Fruit.

The crop was graded to conform with the three Ministry grades, except in the third season, when Grades I and II were combined. Inspection of the yield data showed no marked effect of treatment on Grade II fruit. For this reason a statistical analysis was made of Grade I and total crop only. A summary of the main effects on crop yield is given in Table II.

TABLE II.
YIELDS OF FRUIT.
(Means (27 plots) in lb./plot. Original number=60 plants/plot).

Treatments Year and grade	N_1	N_2	N_3	F	P_0	P_1	P_2	F	Sig. diff.	K_0	K_1	K_2	F
1956													
Grade I	29.7	28.1	27.9	N.S.	26.2	28.2	30.3	*	2.61	27.9	29.2	27.7	N.S.
Total	47.3	45.6	47.0	N.S.	43.8	46.4	49.8	*	4.48	46.4	47.8	45.8	N.S.
1957													
Grade I	45.0	44.6	43.3	N.S.	45.2	43.6	44.0	N.S.	—	42.5	45.1	45.2	*
Total	70.2	71.0	70.8	N.S.	71.2	69.9	70.9	N.S.	—	70.0	71.6	70.4	N.S.
1958													
Grade I and II	87.0	87.5	88.0	N.S.	86.4	87.0	89.2	N.S.	—	86.6	87.7	88.2	N.S.
Total	90.4	91.6	92.5	N.S.	90.5	90.6	93.5	N.S.	—	90.2	91.6	92.7	N.S.

F=Variance ratio. *=Sig. at 5% level. N.S.=not significant.

1956.

Main Effects. There was a small but significant positive effect of phosphate on Grade I and total crop. Although the trend was linear, only P_2 - P_0 was significant (5% level).

There was no significant response to nitrogen or potassium.

Interactions. The NP interaction was significant (5% level) for Grade I and total crop.

	Grade I (means)			Total crop (means)		
	P_0	P_1	P_2	P_0	P_1	P_2
N_1	24.6	33.5	30.0	40.1	53.2	48.4
N_2	28.0	24.4	29.9	46.9	40.9	49.1
N_3	26.0	26.7	31.2	44.5	45.0	51.7
	Sig. diff. (5%)=4.53			Sig. diff. (5%)=7.78		

1957.

Main Effects. Potassium had a small but significant effect (5% level) on Grade I fruit only.

There was no significant response to nitrogen or phosphorus.

Interactions. Not significant.

1958.

Main Effects and Interactions. Not significant.

Leaf Analysis.

The main effects of treatments on leaf nutrient status, at the fruiting stage only, are given in Table III.

TABLE III.
LEAF NUTRIENT STATUS AT FRUIT-RIPENING STAGE.
(Mean values (27 plots) as % dry matter).

Sampling date	Total N			F	Sig. diff.	Total P			F	Sig. diff.	Total K			F	Sig. diff.
	N ₁	N ₂	N ₃			P ₀	P ₁	P ₂			K ₀	K ₁	K ₂		
21.6.56 1st season	2.60	2.66	2.72	***	0.090	0.24	0.24	0.24	N.S.	—	1.46	1.48	1.54	**	0.082
18.6.57 2nd season	2.79	2.86	2.90	***	0.083	0.23	0.24	0.24	N.S.	—	1.56	1.66	1.70	***	0.085
3.7.58 3rd season	2.71	2.83	2.93	***	0.100	0.18	0.19	0.20	***	0.010	1.10	1.28	1.40	***	0.092

F = Variance ratio. ** = Sig. at 1%. *** = Sig. at 0.1% level.

1956.

Main Effects. Significant positive effects, due to increased rates of nitrogen (at 0.1% level) and potassium (at 1% level). No response to phosphate.

Interactions. Not significant.

1957.

Main Effects. Significant positive effects due to increased rates of nitrogen (0.1% level) and potassium (0.1% level). No response to phosphate.

Interactions. A significant NP interaction (1% level).

	Nitrogen as % leaf dry matter		
	P ₀	P ₁	P ₂
N ₁	2.86	2.73	2.77
N ₂	2.81	2.92	2.86
N ₃	2.91	2.90	2.89

Sig. diff. (1%) = 0.064

1958.

Main Effects. Significant effects due to increased rates of nitrogen (0.1% level), phosphate (0.1% level) and potassium (0.1% level).

Interactions. Not significant.

Discussion.

It is clear from these results that a virus-free stock of strawberry plants can produce good yields of fruit for three cropping seasons in a soil relatively low in available phosphate, and with low rates of nitrogenous fertilizer, provided that the potassium supply available from soil or fertilizer is adequate, and pest and disease control is good. The relative lack of response to fertilizers in this experiment is explained by the leaf analysis figures given in Table III.

Previous work (3) has shown that a nitrogen concentration of 2.5 to 2.7% in the leaves (dry matter), at the fruiting stage, is sufficient for good yields of high grade fruit (assuming no other limiting factors). At this nitrogen level the plants may appear slightly nitrogen deficient, but the yield is not affected. Too much nitrogen results in over-vigorous plants and increased losses due to Botrytis. In the present experiment the leaf nitrogen concentration of N_1 -plants ranged from 2.6 to 2.8% over the three seasons; values that are regarded as sufficient. Increased rates of nitrogenous fertilizer raised the leaf-nitrogen status significantly without enhancing the yield of fruit. From these results it should *not* be inferred that 1 cwt. per acre of ammonium sulphate is the optimum nitrogenous dressing for strawberries, but only that it was sufficient under these experimental conditions to maintain an adequate nitrogen status within the plant.

The available soil phosphorus was low, and the plants showed a small but significant response to phosphate in the first cropping season, but not in the second and third seasons. Leaf analysis showed no significant effects due to increased rates of phosphate in the first two seasons, so the effect on yield may have been due to enhanced growth in 1954, although this difference was not sufficient to be observed.

Previous experiments (3) have shown that a leaf-phosphorus concentration ranging from 0.24 to 0.28% of dry matter, at the fruiting stage, is sufficient for high yields of fruit. The leaf-phosphorus status of the P_0 plants fell just within this range, hence the lack of response to phosphatic fertilizer. In the third cropping season, possibly owing to a slightly later sampling date and a heavy crop, the leaf-phosphorus status of all plants was near the threshold level.

The potassium status of the soil, as expressed in terms of exchangeable potassium, was reasonably satisfactory, and this would account for the lack of response to potassic fertilizers. Furthermore, the leaf-potassium status of K_0 -plants in the first two seasons was sufficient (3). There was a significant increase in leaf-potassium due to increased dressings of potassic fertilizer but, apart from a small effect on Grade I fruit in the second season, this was not accompanied by an enhanced crop yield. The later sampling date in the third season, and the heavy crop, probably accounted for the lower leaf-potassium values in that year.

In the absence of non-nutritional limiting factors (including water supply) the response to applied fertilizers is governed by the nutritional status of the crop (as indicated by leaf analysis), and by the fixing power of the soil for the applied nutrients.

When optimum, or sufficiency, levels have been established for each crop at definite physiological stages of growth, then the ideal approach to manuring would be first to determine the current nutritional status of the

crop, and then to adjust the rate, time and method of application of nutrients so as to maintain the nutritional status of the crop as near as possible to the sufficiency levels.

These results illustrate quite clearly that for a correct interpretation of the results of a manurial experiment, assuming that the only limiting factors are nutritional, it is essential to know the nutritional status of the crop, and for this purpose leaf analysis is a reliable guide.

Acknowledgments.

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SPRAY APPLICATION PROBLEMS: XLVI. MERCURY RESIDUES ON COFFEE LEAVES AND BERRIES IN RELATION TO THE CONTROL OF BERRY DISEASE.

By J. A. PICKARD and J. T. MARTIN.

The Coffee Berry Disease Unit of the Department of Agriculture, Kenya, under the direction of Dr. J. Nutman, has investigated amongst other measures the value of mercury sprays for the control of the disease caused by *Colletotrichum coffeanum* Noack on varieties of *Coffea arabica* in East Africa. The control of the disease was satisfactory, but phytotoxic effects were seen in 1957. The damage appeared as a severe leaf scorch four to five months after the last application of the fungicide. Later, other symptoms affecting the growth of the shoots and leaves of *C. arabica* were observed (1). Other matters of importance were the extent to which the whole berries were likely to be contaminated with mercury, and the risk that the mercury might penetrate within the beans and survive the roasting process to contaminate infusions made from them. The residue questions were referred to Long Ashton and analytical work was undertaken on samples of whole berries and of hulled, roasted and ground beans sent by air-mail from Kenya. Tests were also made on leaves to ascertain the degree of retention by the plant of mercury from the spray treatments.

Experimental.

Materials and treatments.

Whole berries were obtained from trees sprayed to run-off with a proprietary phenylmercuric acetate preparation at $2\frac{1}{2}$ lb. per 200 gal. per acre (0.003% Hg in the diluted wash). The treatments were as follows:

Treatment A. Six applications during late February to early March 1957 covering the developmental stages of flower differentiation to small fruitlet.

Treatment B. Four applications from mid-June to mid-August 1957 during which the fruits attained their maximum green size.

Treatment C. Three applications from early September to mid-October covering the commencement of fruit maturation.

Treatment ABC. Thirteen applications comprising A, B and C.

Leaf samples were obtained from trees receiving treatment ABC. They consisted of (a) leaves that were present during the spraying period and (b) leaves which emerged after the spray programme was completed.

Samples of leaves and berries were obtained from untreated plots.

To determine the location of any mercury contamination and its fate in the roasting process and in preparing the beverage, assays were made on the whole berries from plants receiving treatments B, C and ABC; on the seeds and endocarp separately (berries from treatments A and ABC); on the hulled, roasted and ground beans (from treatments A and ABC); and on coffee infusions made from the hulled roasted and ground beans (from treatments A and ABC).

Methods of analysis.

Whole berries; endocarp; seeds; hulled, roasted and ground beans before and after infusion; and leaves. The method of Klein (2) as amended by Abbott & Johnson (3) was used, with minor modifications. Carbon tetrachloride was used as solvent because it gave less emulsification trouble than chloroform in the extraction of the mercury from the acid digest, and the excess of dithizone was extracted with dilute ammonia from the carbon tetrachloride solution of the dithizonate. The colour of the mercury complex was measured at 490 m μ .

Infusions. The recovery of mercury from the infusions presented some difficulty. The concentration of the liquid in a rotary evaporator under reduced pressure at 40°C. led to an 86 per cent. loss of added mercury. Tests with a number of ion exchange resins showed that De-acidite G would retain the mercury satisfactorily and the following method was adopted. The infusion was prepared by adding 300 ml. of boiling water to 20 g. of hulled, roasted and ground beans and allowing to stand for five minutes. The solution was filtered hot through a No. 54 Whatman paper, 5 ml. of hydrochloric acid were added to convert the mercury to the ionic form and the solution was allowed to stand until cool. It was then passed through a 7 $\times$ 2 cm. column of De-acidite G at the rate of 1 to 2 ml. per minute. The column was washed with 50 ml. of water, 50 ml. of ethyl alcohol and a further 50 ml. of water. The mercury was removed from the resin with 350 ml. of 30 per cent. nitric acid, the eluate was neutralized to litmus with concentrated ammonia solution, 5 ml. of hydrochloric acid followed by 10 ml. of hydroxylamine hydrochloride were added, and the dithizonate was extracted and measured in the usual way.

Recoveries of added mercury. Known amounts of mercury, as mercuric chloride in nitric acid solution, were added before digestion to whole berries, endocarp, seeds, roasted ground beans and leaves. Mercury was added as phenylmercuric acetate in aqueous solution to infusions before the addition of the hydrochloric acid. The recoveries obtained are given in Table I.

TABLE I.
RECOVERIES OF ADDED MERCURY.

	Hg added, μ g.	Hg found, μ g.	Recovery per cent.
Whole berries	30	25.0	83
	30	23.2	77
Endocarp	30	23.5	78
	40	36.0	90
Beans	30	24.7	82
	40	36.2	91
	50	45.1	90
	46	40.5	88
Infusions	46	40.6	88
	50	47.4	95
	40	38.8	97
Leaves	40	37.8	95
	20	19.1	96

The best recoveries were obtained from the leaves, which were readily acid-digested. Prolonged acid treatment was needed to destroy the organic matter of the beans, some of the fat resisting oxidation. In view of the difficulties met, the recoveries of mercury from all the materials were regarded as satisfactory.

Analysis of the samples.

Mercury content of whole berries. The results of the analysis of whole berries as p.p.m. Hg corrected for control values are given in Table II. The treated sample 1 and the control sample 1 were taken from the relevant plots of one block in the field experiment and the treated and control samples 2 to 8 were similarly related.

TABLE II.
MERCURY CONTAMINATION OF WHOLE BERRIES.

Sample	Treatment			Control
	B	C	ABC	
1	1.04	0.09	0.92	0.03
2	1.03	0.02	1.91	—
3	0.47	—	0.75	0.05
4	—	0.24	0.91	—
5	0.64	0.22	1.32	—
6	—	—	0.96	—
7	0.75	—	0.40	0.04
8	0.96	0.23	1.02	0.04
<i>Mean</i>	<i>0.81</i>	<i>0.16</i>	<i>1.02</i>	<i>0.04</i>

The treatment in mid-season (B) gave a level of contamination approaching that from all the treatments, the late treatment (C) contributing relatively small deposits. The highest deposit from all the treatments was 1.9 p.p.m. and the average deposit was 1.0 p.p.m. Hg.

Distribution of mercury between endocarp and seed. As the endocarp is removed from the seed before roasting, the distribution of mercury in whole berries from treatment ABC was determined by the examination of endocarp and seed separately (Table III).

TABLE III.
DISTRIBUTION OF MERCURY BETWEEN ENDOCARP AND SEED.

Sample		Hg, p.p.m.	Hg, per cent.
2	Endocarp	1.48	16
	Seeds	2.02	84
4	Endocarp	1.66	34
	Seeds	0.75	66
5	Endocarp	1.65	27
	Seeds	1.23	73
6	Endocarp	1.34	30
	Seeds	0.86	70
<i>Mean</i>	<i>Endocarp</i>	<i>1.53</i>	<i>27</i>
	<i>Seeds</i>	<i>1.22</i>	<i>73</i>

Table III shows that three-quarters of the mercury contaminating the berries occurred in the seeds. The p.p.m. of mercury calculated on the whole berries have been included in Table II.

Mercury content of hulled, roasted and ground beans. Twelve samples of hulled, roasted and ground beans were obtained from plots receiving the early treatment, and eight samples from those given the complete treatment. The results of analysis, as Hg p.p.m., are shown in Table IV.

TABLE IV.
MERCURY CONTENT OF HULLED, ROASTED AND GROUND BEANS.

Sample	Treatment	
	A	ABC
1	0.39	1.16
2	0.55	1.38
3	0.61	0.90
4	—	0.58
5	0.53	0.90
6	0.42	0.74
7	0.69	—
8	0.44	0.83
9	0.88	—
10	0.95	—
11	—	—
12	0.43	—
<i>Mean</i>	<i>0.59</i>	<i>0.93</i>

The values for mercury after roasting the beans are surprisingly high. Assuming a loss in weight of between 12 and 16 per cent. on roasting (taken from the literature) the mean values for the beans from treatment ABC before and after roasting show that this process results in a loss of about one-fifth of the mercury. Roasted, ground beans obtained from a local store showed no mercury contamination.

Mercury contamination of infusions. Infusions of roasted, ground beans from treatments A and ABC were made and analysed by the methods described above. The results, shown in Table V, represent the p.p.m. of mercury originally present in the roasted, ground beans that found their way into the infusions or remained in the solid residues.

TABLE V.
MERCURY CONTENT OF INFUSIONS AND SOLID RESIDUES.

Sample	Treatment			
	A		ABC	
	Infusion	Residue	Infusion	Residue
3	0.09	0.46	—	—
7	—	—	0.16	—
8	0.08	0.41	0.13	0.50
9	0.13	0.78	—	—
10	0.14	0.83	—	—
<i>Mean</i>	<i>0.11</i>	<i>0.62</i>	<i>0.15</i>	—

Table V shows that a relatively small proportion of the mercury present in the ground beans was taken up by infusions made by our method. Assuming that 1 oz. of ground beans is used to make 1 pint of beverage and that this is equivalent to $3\frac{1}{2}$ cups, each cupful would contain approximately $1\text{ }\mu\text{g}$ of mercury.

Mercury deposits on leaves. The results of analysis, allowing for records from the controls, of leaves present during the period of the ABC treatment and of those which emerged subsequent to spraying are shown in Table VI as p.p.m. Hg.

TABLE VI.
MERCURY DEPOSITS ON LEAVES.

Sample	Treated Old	Untreated New	Control	
			Old	New
1	22	0.62	0.23	0.20
2	14	0.49	0.18	0.20
3	15	0.63	0.30	0.20
4	17	—	—	—
5	13	—	—	—
6	14	—	—	—
<i>Mean</i>	<i>17</i>	<i>0.58</i>	<i>0.24</i>	<i>0.20</i>

The average size of the old leaves was 60 sq. cm. and of the young leaves 23 sq. cm. The old leaves showed 17 p.p.m. of mercury, but the extent to which weathering of the deposit may have taken place could not be assessed. The presence of a significant amount of mercury in the young leaves which emerged after spraying was completed is of some interest. Bock, Robinson & Chamberlain (1) when describing the damage to coffee trees caused by mercury referred to the bunched, rosetted appearance of the youngest leaves at the tips of the shoots. The damage was shown to be associated with zinc deficiency induced by the mercury; the analytical data above indicate that mercury may have been present in the young damaged leaves. Work on apple trees has shown that mercury is absorbed by the leaf, some being held by the cellular and some by the fibrous tissue (4), and that while mercury applied to shoots is mostly held by the bark, some is also present in the internal tissues. These observations point to the absorption of mercury by the plant and its translocation to new emerging foliage: this merits further examination.

Although experimental control of the disease in Kenya was obtained, the damage caused to the plant, apart from the contamination of the berries, has made it impossible for the treatment to be adopted as a remedial measure.

Summary.

Whole berries from coffee trees receiving thirteen applications of a fungicidal mercury spray during the period of fruit development showed an average mercury contamination of 1 p.p.m. Much of the mercury present in the berries was located in the seeds. Little of the mercury was lost on roasting the hulled beans. Infusions made from the hulled, roasted and ground beans showed little contamination, most of the mercury being retained by the solid residues.

An examination of mercury deposits on coffee leaves indicated the possibility of the translocation of mercury from sprayed foliage to newly emerging leaves.

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SPRAY APPLICATION PROBLEMS : XLVII. THE DETERMINATION OF RESIDUES OF GAMMA-BHC.

By J. A. PICKARD.

The method used by us for the determination of residues of γ -1:2:3:4:5:6-hexachlorocyclohexane is based upon that proposed by Schechter and Hornstein (1) in which the compound after extraction from plant material is converted to benzene, the benzene is nitrated, and the *m*-dinitrobenzene is determined colorimetrically. Various modifications have been introduced. Methylene chloride is virtually free of aromatic compounds and so is preferred as solvent for the extraction of the plant material. The extract is purified by passage through alumina of Brockman activity I. An apparatus similar to that proposed by Hancock and Laws (2) is employed for the dechlorination process, carbon dioxide gas being led from a cylinder through a side tube into the reaction flask instead of using malonic acid. An alternative colour reaction for *m*-dinitrobenzene using methyl ethyl ketone and tetraethylammonium hydroxide avoids the use of immiscible reagents and gives immediate colour formation.

The Method of Analysis.

The details of the method, as used for the determination of gamma-BHC in flour, are as follows :

Reagents. Careful purification of some of the reagents is essential if high blank values are to be avoided. Acetic acid was refluxed with zinc powder and then with chromium trioxide and fractionated. *Isobutyl* methyl ketone was extracted with portions of 50 per cent. potassium hydroxide solution until the extract was colourless, dried over anhydrous sodium sulphate and fractionated with the collection of the portion boiling at 115-116°C. Methylene chloride and methyl ethyl ketone were fractionated to give portions boiling at 39.5-40.5°C. and 79.5-80.5°C. respectively. The tetraethylammonium hydroxide reagent was prepared by diluting 8 ml. of the 25 per cent. material to 100 ml. with methyl alcohol. Aluminium oxide for chromatographic analysis was heated at 600°C. for 12 hr. and stored over phosphorous pentoxide or in sealed glass tubes. Cotton wool was extracted with methylene chloride. Polythene tubing was used for glass connections.

The alumina column was prepared by transferring a slurry of 10 g. of alumina in methylene chloride to a 25×1.5 cm. glass tube and washing with more solvent. A tap at the lower end of the tube controlled the elution rate to 1–2 ml. per min.

Extraction and alumina treatment. 20–25 g. of flour in a thimble were extracted in a Bolton apparatus for 3 hr. with a minimum of methylene chloride. The cold extract was passed through an alumina column and washed through with 10, 10 and 5 ml. portions of methylene chloride. The eluate was reduced to 1 ml. volume by drawing a current of dry air over its surface at $30-35^{\circ}\text{C}.$, and the remaining solvent was removed by rotation of the flask at room temperature. The residue was dissolved in 10 ml. of warm acetic acid and transferred to the reaction flask of the dechlorination apparatus, through a small cotton wool filter, with 4, 3, and 3 ml. portions of acid.

Dechlorination. 4 g. of zinc were added, and the mixture gently boiled for $2\frac{1}{2}$ hr. with passage of a steady stream of carbon dioxide to convey the benzene formed in the reaction to the nitration mixture (4 ml. of a mixture by volume of 1 part of nitric acid sp.gr. 1.42 and 5 parts of concentrated sulphuric acid). Tap water was passed through the condenser. The nitration mixture was then diluted with 25 ml. of water and transferred to a flask, the nitration vessel and delivery tube being washed with a further 30 ml. of water. The solution was neutralized to litmus with 25 per cent. potassium hydroxide solution and transferred to a separating funnel, adjusting the volume to about 100 ml. with water. 20 ml. of *isobutyl* methyl ketone were added, the mixture shaken for one minute, allowed to separate and the aqueous layer discarded.

Colour formation. 9 ml. of methyl ethyl ketone were added to an aliquot (10 ml. or less) of the *isobutyl* methyl ketone solution and the solution mixed. 1 ml. of tetraethylammonium hydroxide reagent was added and the volume was adjusted, if necessary, to 20 ml. with *isobutyl* methyl ketone. The optical density was measured within 5 min. at $565\text{ m}\mu$ in a 2 cm. cell.

The values obtained for the calibration curve, determined on known amounts of gamma-BHC added to flour extracts, and for the recoveries of gamma-BHC added in methylene chloride solution to samples of uncontaminated flour before extraction are shown in Table I.

TABLE I.
OPTICAL DENSITY VALUES AND RECOVERIES OF GAMMA-BHC FROM FLOUR.

$\mu\text{g. added}$	Optical density*	$\mu\text{g. recovered}$
40	0.135	38, 35
60	0.195	—
80	0.275	82, 60
100	0.330	101, 95, 97, 107
120	0.405	126
140	0.477	157, 120
160	0.540	168
180	0.595	—
200	0.675	203

* Since 10 ml. aliquots were taken, these values were given by half the quantities of gamma-BHC added.

The optical densities recorded in determinations on acetic acid solutions containing known amounts of gamma-BHC agreed closely with those obtained

when the compound was added to flour extracts. For this reason, the occasional low values recorded in Table I were thought to be due to the incomplete extraction of the gamma-BHC from the flour. Tests on pure gamma-BHC and pure *m*-dinitrobenzene showed that 85 per cent. conversion was obtained. Values obtained on reagents alone were equivalent to 2.6 μ g., and on 20g. samples of uncontaminated flour to 4.6 μ g. of apparent gamma-BHC.

The method is applicable to the determination of gamma-BHC deposits on foliage. Surface deposits may be removed by washing whole leaves with methylene chloride and absorbed deposits by methylene chloride extraction of the powdered washed leaves after air-drying them at normal pressure over silica gel. Some loss may occur in the process of drying the leaves. In the analysis of non-fatty materials, the addition of a little liquid paraffin before removing the methylene chloride is recommended.

Summary.

A method for the determination of residues of gamma-BHC is described.

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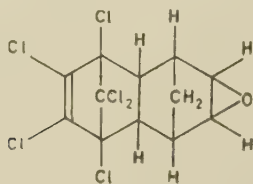
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SPRAY APPLICATION PROBLEMS: XLVIII. THE DETERMINATION OF MICROGRAM QUANTITIES OF DIELDRIN.

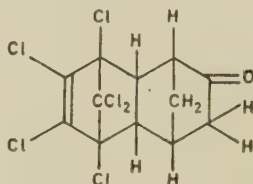
By E. J. SKERRETT and E. A. BAKER.

Methods for the estimation of spray residues of dieldrin, a commercial product containing at least 85% HEOD (1) (1:2:3:4:10:10 hexachloro-6:7-epoxy-1:4:4a:5:6:7:8:8a-octahydro-*exo*-1:4-*endo*-5:8-dimethanonaphthalene), include the determination of the chlorine in the molecule (2, 3) after removal of interfering material, infra-red absorption (4), or the formation of a colour by heating with *p*-nitrophenol (5). However, the most widely used colorimetric method is that due to O'Donnell *et al.* (6). In this method the epoxide ring of dieldrin (I) is opened by treatment with a mixture of acetic anhydride and hydrobromic acid to yield a 7-bromo-6-acetate derivative. This is then reduced with zinc and glacial acetic acid to yield aldrin and its partially dechlorinated analogues. An adduct with phenyl azide is then prepared and coupled with diazotised 2:4-dinitroaniline. The resulting deep red colour is measured in concentrated sulphuric acid solution.

It was hoped that it might be possible to develop an alternative to the phenyl azide method to avoid the necessity of working in a darkened room and to obviate the variable sensitivity of the reagent.



I



II

In the chemistry of the steroids boron trifluoride has been used to catalyse the isomerization of epoxide rings to a corresponding ketone (7, 8, 9). If a similar re-arrangement were to occur in the case of dieldrin then a polycyclic ketone would result which would have the structure shown in II. This reaction could then be made the basis of a colorimetric method by preparation of a 2:4-dinitrophenylhydrazone whose colour could be intensified by the action of alkali (10).

Experimental.

Commercial samples of dieldrin were purified by boiling with methanol, filtering the hot solution from the insoluble residue, and collecting the crystals deposited on cooling. Three such treatments gave material m.p. 176°C. which was used in the development of the method.

In the preliminary experiments a commercial specimen of the boron trifluoride-ether complex was used but, while it was satisfactory in establishing that the transformation appeared to occur in the postulated manner, the reagent gave rise to large blanks. It was found necessary to prepare the boron trifluoride-ether complex under rigorously controlled conditions in order to reduce the blank values from this cause to an acceptable level. Traces of water had to be removed from the solvents used in the isomerization stage, and the A.R. grade 2:4-dinitrophenyl-hydrazine recrystallized twice before the blank was reduced to a minimum.

House and Wasson (11) had shown that in the steroids the use of ether as solvent could occasionally lead to side-reactions in the isomerization of epoxides (cf. House (12)) and for this reason benzene was used as the reaction medium. While acetic acid was used initially to dissolve the residual ketone after isolation from the isomerization mixture, it gave rise to a small but persistent blank which could not be reduced by any purification procedures applied to the solvent. The use of ethanol as an alternative successfully obviated this trouble. After removal of excess 2:4-dinitrophenylhydrazine the colour of the 2:4-dinitrophenylhydrazone in benzene solution was intensified by the use of tetraethylammonium hydroxide (13) and measured with a Unicam SP600 at 440 m μ .

Method.

Reagents.

Boron trifluoride-ether complex : Pass a slow stream of gaseous boron trifluoride into ether at -70°C . with gentle agitation until no further boron trifluoride is absorbed. Store the reagent at 0°C . with precautions against the ingress of moisture and discard it as soon as it shows any significant yellowing.

Benzene : Reflux A.R. grade benzene with 0.5% boron trifluoride-ether complex for 4 hours. Cool, wash with water three times, once with 10% sodium hydroxide solution, and finally with water. Add 1g./l. of 2:4-dinitrophenylhydrazine and 1g./l. of trichloroacetic acid (13) and heat for 6-8 hours using a Dean and Stark trap to remove water. Fractionally distil, then re-fractionate after refluxing for 8 hours with potassium.

Ether : Prepare in the manner described for benzene.

Ethanol : Fractionate commercial absolute alcohol from 1g./l. of 2:4-dinitrophenylhydrazine and 0.2 ml./l. of concentrated hydrochloric acid.

2:4-Dinitrophenylhydrazine solution : Prepare freshly each day by dissolving 10 mg. of twice recrystallized (benzene) 2:4-dinitrophenylhydrazine in 5 ml. 20% v/v sulphuric acid. Extract the solution with 0.1 ml. of benzene immediately before use.

Tetraethylammonium hydroxide solution : 25% w/v.

Hydrochloric acid : sp. gr. 1.18.

Potassium hydroxide solution : 10N.

Procedure.

To a solution containing 0-100 μg . of dieldrin in 5 ml. of benzene add 1 ml. of a freshly prepared 9:1 mixture of benzene and boron trifluoride-ether complex. Heat under anhydrous conditions for 30 minutes at 78°C and then cool in ice-water. Slowly add 20 ml. of saturated sodium bicarbonate with careful cooling and transfer to a separating funnel with the aid of 5 ml. of benzene. Shake the funnel cautiously, venting the evolved carbon dioxide at frequent intervals, until no more carbon dioxide is evolved. Discard the aqueous layer and wash the benzene solution with water, again discarding the aqueous phase. Dry the benzene with sodium sulphate and evaporate the solution and washings to dryness in a water-bath. Warm the residue slightly with 0.5 ml. ethanol to dissolve it, and then allow the solution to stand for 15 minutes with 0.1 ml. of the 2:4-dinitrophenylhydrazine solution. Transfer the mixture to a separating funnel with 5 ml. of benzene, and wash it once with 10 ml. of concentrated hydrochloric acid, once with 5 ml. of 10N sodium hydroxide, again with 10 ml. concentrated hydrochloric acid, and finally with 20 ml. of water. Dry the benzene with anhydrous sodium sulphate and make up to a volume of 7.5 ml. in a measuring cylinder. Add 2.5 ml. of ethanol and 1 drop of 25% tetraethylammonium hydroxide solution and mix thoroughly. Measure the resultant colour in a 1 cm. cell in a Unicam SP600 at $440\text{ m}\mu$ after 1 minute.

The quantity of dieldrin can then be interpolated from the standard graph (Fig. 1).

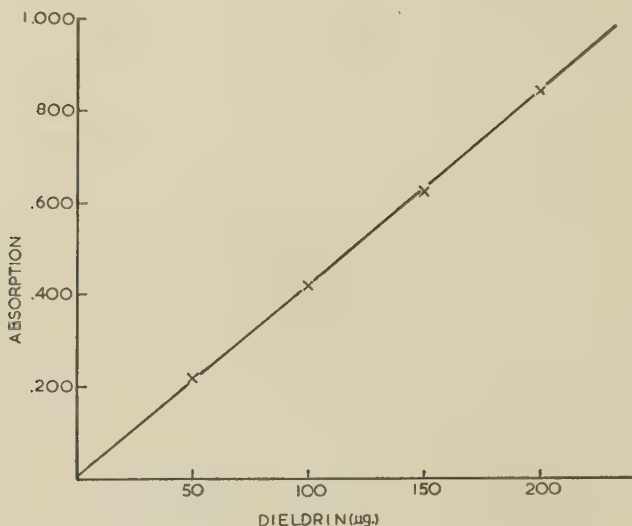


Fig. 1. Calibration curve for determination of dieldrin.

Determination of Endrin.

Endrin is a spatial isomer of dieldrin and it was hoped that it would be possible to utilize the same technique for the colorimetric determination of that insecticide. Although the use of the reaction procedure described above gave a significant colour reaction, it was found necessary to reduce to 0.65 ml. the volume of the benzene solution of the boron-trifluoride-etherate and to reduce the reaction temperature to 40°C. Even so, the method was only half as sensitive as that for dieldrin, the absorption for 100 μg. of the insecticide being 0.365 in a 2 cm. cell. The apparent disparity in sensitivity is presumably due to side-reactions; present work is aimed at developing an alternative method of greater sensitivity.

Summary.

A method is described for the colorimetric determination of small quantities of dieldrin. With slight modification the method is applicable to endrin but the sensitivity is approximately halved.

Acknowledgments.

The authors thank Professor Kearns and Dr. J. T. Martin for their interest in the work, and Messrs. Imperial Smelting Corporation Limited for the gift of a cylinder of gaseous boron trifluoride.

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SPRAY APPLICATION PROBLEMS: XLIX. THE DETERMINATION OF ROTENONE IN LONCHOCARPUS.

By D. V. RICHMOND.

Before the last war, extracts of derris root (*Derris elliptica* Benth. and *D. malaccensis* Prain) from Malaya and other tropical regions were widely used as horticultural sprays. Derris also became the standard remedy for the control of warble fly in cattle. With the outbreak of hostilities, supplies of derris root were much reduced, and lonchocarpus root (*Lonchocarpus utilis* A. C. Smith and *L. urucu* Killip and Smith) from South America took its place. Today, lonchocarpus forms the bulk of the insecticidal root used in commerce.

Lonchocarpus root is very similar chemically to *D. elliptica*. The chief insecticidal component, rotenone, occurs to the extent of about 5 per cent. of the air-dry root, along with other compounds of the rotenone class. Colorimetric (1, 2, 3, 4) or absorptiometric (5) procedures are available for the determination of rotenone, but the method most widely used in commerce depends upon the separation from the root extract of a crystalline rotenone-carbon tetrachloride solvate (6, 7, 8). Other components of the extract may suppress the crystallization of the solvate, and this was overcome in earlier work on derris extract by a preliminary purification process involving the extraction with dilute alkali of phenolic and other materials (9). Interest has recently been revived in the analysis of rotenone-bearing roots, as evidenced from discussions with visitors from overseas, and more recently, by the institution of collaborative analytical work in this country. Comparative analyses of lonchocarpus root and resin were therefore made by the direct crystallization method (7) as adopted by the British Veterinary Codex, 1953, and by the crystallization of the solvate after the purification of the extract. This note records the alkali extraction procedure used at Long Ashton and the results of a chromatographic examination of the resin.

Experimental.

The materials examined consisted of powdered Peruvian lonchocarpus root and a lonchocarpus resin.

Method of Analysis.

The root (20 g.) was extracted by hot percolation with benzene for 3 hr., the cooled solution was adjusted to 50 ml. with benzene and diluted with an equal volume of ether. The resin (5 g.) was dissolved in 100 ml. of a 50:50 benzene-ether mixture.

The benzene-ether solution was extracted successively with 50 ml. portions of 2, 5 and 5 per cent. potassium hydroxide solution, using gentle rotation in a separating funnel. Each alkaline layer was removed as quickly as possible and an excess of water was added to reduce the concentration of the residual potash in the benzene-ether solution. The alkaline extract was washed with benzene-ether, which was added to the main solution. This was then just acidified to litmus paper by the addition, drop by drop, of dilute hydrochloric acid with shaking. The benzene-ether layer was well washed with water, dried over anhydrous sodium sulphate and the solvent removed by distillation and finally under reduced pressure.

The resin obtained was dissolved by warming in five times its weight, in ml., of carbon tetrachloride saturated with rotenone at 0°C. and kept at 0°C. overnight. The solvate was filtered off using pre-cooled apparatus, washed with a minimum of cold carbon tetrachloride saturated with rotenone at 0°C., dried at 40°C. for 1 hr., and weighed.

The content of rotenone in the solvate was calculated from its optical rotation in benzene. A 4 per cent. solution of the pure solvate in benzene shows $[\alpha]_D^{20} -166^\circ$.

Analytical results.

The values recorded for rotenone in the root and resin by the two methods were as follows :

Root : *Direct crystallization of solvate* : range of four determinations, 5.6 to 5.8 per cent. ; mean value 5.7 per cent. rotenone.
Crystallization of solvate after alkali treatment : range of three determinations, 6.2 to 6.3 per cent. ; mean value 6.2 per cent. rotenone.

Resin : *Direct crystallization of solvate* : range of seven determinations, 32.4 to 33.8 per cent. ; mean value 33.1 per cent. rotenone.
Crystallization of solvate after alkali treatment : range of four determinations using benzene-ether (50:50), benzene or chloroform as solvent 36.2 to 37.2 per cent. ; mean value 36.7 per cent. rotenone.

The results show that appreciably higher values for rotenone in both root and resin were given by the crystallization of the solvate after alkali treatment than by the present standard method of direct crystallization of the solvate.

Chromatographic examination of lonchocarpus resin.

The possibility was examined of purifying the resin by passage through an absorbent before the crystallization of the solvate. With benzene as solvent, cellulose was found unsatisfactory as an absorbent, while silica gel and magnesium oxide gave incomplete recoveries of rotenone. Sepiolite gave a good recovery, and alumina of activity grade V also proved to be an efficient absorbent, removing substances inhibiting the crystallization of the solvate without loss of rotenone. Alumina of higher activities than IV or V produced purer solvates but the recoveries were low.

When pure rotenone, in benzene solution, was passed through freshly prepared grade V alumina (80 × 16 mm. column) the recovery was 94 per cent. On cooling the solution to 6°C. before passage through the column, the recovery rose to 96.5 per cent. Of the 3.5 per cent. loss of rotenone, 2.3 per cent. occurred on the column and 1.2 per cent. during the formation of the solvate.

Analyses of benzene extracts of the root and benzene solutions of the resin, using grade V alumina columns varying from 70 to 200 × 16 mm. in size and 150 ml. of benzene for elution, gave the following results :

Root : *Crystallization of solvate after alumina treatment* : range of five determinations 6.6 to 6.7 per cent. ; mean value 6.7 per cent. rotenone.

Resin : *Crystallization of solvate after alumina treatment* : range of three determinations 38.0 to 38.7 per cent. ; mean value 38.3 per cent. rotenone.

The results show that the alumina column treatment yielded 8 per cent. more rotenone in the analysis of the root and 4 per cent. more in tests on the resin, than the alkali washing procedure, which in turn was more efficient than the direct crystallization method. A Sepiolite (100 mesh) column gave a value of 6.7 per cent. rotenone in the root. Further work traced the slightly lower recoveries of rotenone following alkali extraction to decomposition during the washing procedure.

Summary.

A comparison of methods for the determination of rotenone in lonchocarpus root and resin showed that the values obtained by a standard method of direct crystallization of solvate were appreciably lower than the true contents and pointed to the desirability of adopting a described alternative method of analysis.

Acknowledgment.

I am indebted to Dr. J. T. Martin for suggesting the problem and for helpful discussion.

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INVESTIGATIONS ON PLANT CUTICLES.

By J. T. MARTIN.

The objectives of the investigations on plant cuticles have already been described in detail (1). At the present stage assessments are being made, on a quantitative chemical basis, of the natures of the cuticles of a wide range of plants in order to gain a better understanding of the parts the cuticles may play in the retention, penetration and occasional damaging effects of spray chemicals and in host-parasite relationships. Our interest centres chiefly on leaves, but some work on fruits has also been carried out. Methods have been devised for the separation and fractionation of the waxy coverings of leaves and fruits and for the isolation of cuticular membranes and the determination of their cutin contents (1, 2). The results of further collaborative work with these methods, carried out in 1958 by Miss M. F. Roberts, R. F. Batt, D. V. Richmond and the author, are summarized in this report.

Experimental.

Isolation and examination of cuticular membranes.

Wax-free cuticular membranes, composed largely of cutin, were isolated from the leaves of a number of plants, including apple, chrysanthemum, ground ivy, laurel, rhododendron and Euonymus and from the fruits of apple, tomato and black currant. Circular leaf discs, 3 sq. cm. in size, were extracted successively with ether and methyl alcohol to remove waxes, pigments and other materials. The discs were then gently refluxed with an ammonium oxalate (1.6 per cent.)—oxalic acid (0.4 per cent.) solution for varying periods until the membranes separated or could easily be peeled off. The membranes from the upper and lower leaf surfaces were recovered separately. Apple, tomato and black currant fruit membranes were readily obtained by similar treatment of cylindrical plugs, composed of cuticle and a little flesh, punched from the fruits. The membranes from the leaves or fruits were cleaned by refluxing successively with 1.25 per cent. sulphuric acid and 0.2 per cent. potash solution, washed with water and stored in ethyl alcohol.

Examination was made of the membranes isolated by the above method from leaves showing pronounced cuticles, e.g. apple and Euonymus. Photomicrographs of the surfaces of the leaf membranes showed an apparent cellular structure (Figs. 1, 2 and 3, Plates XI and XII). Impressions of stomata were conspicuous in the membranes from the lower leaf surfaces (Figs. 2 and 3). Transverse sections showed that the membranes consisted of a layer containing the cutin with partially occluded remnants of the epidermal cells (Fig. 4). In freeing the membranes, the oxalate solution had clearly effected a break between the epidermal cells and mesophyll tissues. Immersion of the membranes for a short period in cuprammonium solution removed the cellular tissue, leaving an unbroken sheet which showed clearly-defined cuticular ridges that had occupied the depressions between the epidermal cells.

Some leaves, e.g. of red beet, cauliflower, tomato and banana, showed feebly-developed cuticular membranes, and the separation of these without damage was more difficult. The membranes could, however, be successfully isolated by a variation of the method. Small leaf discs taken from between the veins were extracted successively with methyl alcohol, oxalate solution,

PLATE XI.

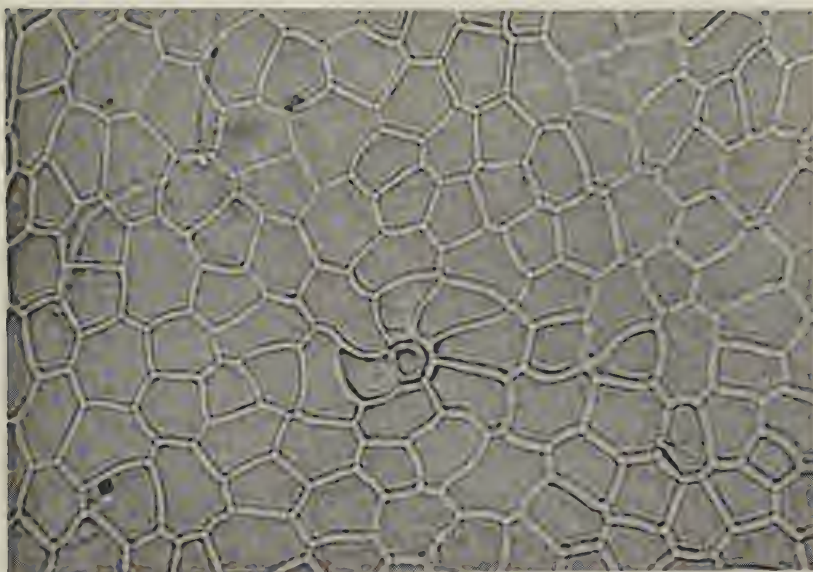


Fig. 1. Surface view of cuticular membrane from upper surface of apple leaf.



Fig. 2. Surface view of cuticular membrane from lower surface of apple leaf.

PLATE XII.



Fig. 3. Surface view of cuticular membrane from lower surface of *Euonymus* leaf.

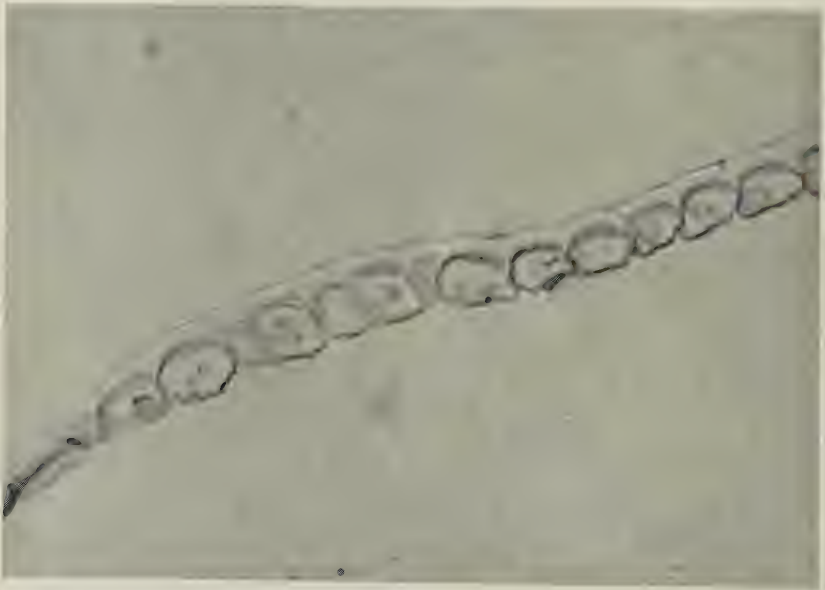


Fig. 4. Transverse section of cuticular membrane from upper surface of *Euonymus* leaf.

dilute potash solution and dilute sulphuric acid. All that remained of the discs after these treatments were the cuticular membranes held together by the cellulosic walls of the mesophyll cells. Treatment with cuprammonium solution removed the cellulose to liberate the membranes. The cuticular membranes of the red beet leaf were extremely fragile and showed no apparent cellular structure.

The composition of the cuticular membrane is of some importance in relation to its permeability. Staining with ruthenium red and examination with polarized light showed that pectin was present within the cutin of the *Euonymus* leaf, the pectin being sufficiently embedded in the cutin to resist extraction during the earlier oxalate treatment. Analytical work has indicated that cellulose was also present to a small extent within the cutin layer.

Irregularities were observed in the patterns of the cuticular ridges of the membranes from the apple leaf (Figs. 1 and 2). These were most prevalent in the membranes from the lower leaf surface, were clearly differentiated from the stomata and occurred often in regions near the underlying conducting tissues (Fig. 2). Sections were made to ascertain whether the irregular structures were aberrant stomata or resulted from fractured hair cells. Narrow, thick-walled depressions in the cutin layer were seen, but whether these were pits or pores was not determined.

The cuticles of apple leaves and fruits.

A detailed examination was made of the wax, oil and cutin components of the cuticles of apple leaves and fruits (3). The extent to which phenolic substances occurred in apple leaves and were present in the surface waxy deposits was also investigated. A micro method was devised for the determination of the phenolic compounds in terms of phloridzin.

The separation of the waxy covering from apple leaves was not easy. The rapid immersion of whole leaves in successive portions of ether at room temperature was used, but there was no assurance that the treatment was recovering waxy material embedded within the cutin layer, or that it was not extracting additional material from within the leaf. The analysis of successive ether washings, up to sixteen, showed that most of the waxy material was removed by the first three immersions; further treatments yielded small amounts of material containing appreciable proportions of phenolic compounds. Four immersions of whole leaves in ether were therefore made standard practice for the removal of the waxy covering.

The appearance, in successive extracts, of phenolic substances suggested that these were being slowly leached from within the leaves. The total amount of phenolic material obtained, however, was small in comparison with the large reserve of phenolic compounds within the leaf. Other work indicated that phenolic substances were present on leaf surfaces. Rain films collected from the upper surfaces of apple leaves gave positive reactions for phenolic materials, and analyses of the rain drippings over a period of one month showed that phenolic compounds were lost from the leaves. The loss was greatest during the first few hours of a long period of steady rainfall. These observations point to the migration of phenolic compounds from within the leaf to the surface layers.

Records were taken of the levels of waxy deposits and of internal phenolic compounds in the leaves of Worcester Pearmain apple trees grown in pots

under nutrient deficiency conditions. Nitrogen deficiency resulted in reduced wax formation, while the old leaves suffering from nitrogen, phosphorus or potassium deficiency were particularly rich in phenolic compounds. The lower wax deposits on the nitrogen-deficient leaves may be associated with their reputed susceptibility to damage by some spray chemicals.

The changes in the waxy and cutin components of cuticles of the leaves and fruits of Merton Worcester and Cox's Orange Pippin apple were followed during the period of fruit development from early June to September. The leaves had just attained maximum size when the tests were begun; only leaves from old wood were used. The combined wax and oil fractions of the surface deposits on the leaves remained constant for most of this period at about 20 $\mu\text{g}/\text{sq. cm.}$ of surface. The cutin, determined separately in the upper and lower cuticular membranes, remained constant for both surfaces at about 100 $\mu\text{g}/\text{sq. cm.}$ of surface. No significant differences were found in the cuticles of the leaves of the two varieties.

The surface deposits of wax and oil on the fruits increased markedly, in equal proportions, as the fruits attained full size. Thereafter, the oil fraction tended to increase further and the wax to diminish. The combined wax and oil deposit on the fully-grown fruit was of the order of 200 $\mu\text{g}/\text{sq. cm.}$ of surface. Cutin deposition in the cuticles increased steadily up to the time when the fruits were ripe, when a little under 1000 μg of cutin per sq. cm. of surface was present. The cuticle of the fully-ripe Cox fruits was made up of 140 $\mu\text{g}/\text{sq. cm.}$ of wax, equally divided between the surface deposit and the cutin layer, 370 μg of oil, also partly on the surface and partly embedded in the cutin, 450 μg of acidic (including phenolic) material, mostly present in the cutin layer, and 950 μg of cutin.

The cuticles of other plants.

The cutin contents of the leaf cuticles of black currant, strawberry, banana, tomato, cauliflower and red beet and of the fruit cuticles of tomato and black currant were also examined. Previous tests on laurel, rhododendron and Euonymus leaves (4) had shown that determinations of cutin made on isolated cuticular membranes and on discs of leaf tissue agreed well. In this work, therefore, no attempt was made to isolate the membranes, the analyses being made on leaf discs taken after the removal of the surface waxy deposits.

Cutin in the Malvern Cross black currant leaf increased from 20 $\mu\text{g}/\text{sq. cm.}$ of surface to 90 $\mu\text{g}/\text{sq. cm.}$ between the grape and early green berry stages when the leaf was expanding. Thereafter the cutin level remained constant. The waxy material in the mature black currant leaf was equivalent to about 40 $\mu\text{g}/\text{sq. cm.}$, an oil fraction predominating. Young Royal Sovereign strawberry leaves showed 45 μg of cutin per sq. cm. of surface; the leaves on plants that had earlier been damaged by a systemic insecticidal spray contained half this level of cutin. The waxy deposit, mostly oil, on the new leaves from the damaged plants was also reduced. Comparisons were made of the cutin contents of young and old leaves of Lacatan and Cavendish banana raised at Long Ashton. The young and old Lacatan and the young Cavendish leaves showed 20-25 μg of cutin per sq. cm.; the old Cavendish leaf 45 $\mu\text{g}/\text{sq. cm.}$ Tomato leaflets, taken from the glasshouse, contained 25 μg cutin per sq. cm., and were virtually non-waxy. Young cauliflower and red beet leaves were characterized by low contents of cutin in their cuticles, values of the order of

10 $\mu\text{g/sq. cm.}$ of surface being recorded. The cauliflower leaves showed 40 μg of waxy material/sq. cm., 50 per cent. of which consisted of wax. As the cauliflower leaves grew older, the total amount of waxy deposit, and its content of wax, increased.

Cutin was determined in the cuticles of tomato fruits at different stages of growth. The small green fruits contained 770 μg and the large ripe fruits 1,250 μg of cutin per sq. cm. of surface. As the fruits developed, cutinization progressed inwards, three or four layers of cells being occluded within the cutin layer of the mature fruits. Fully grown black currant fruits showed 130 μg of cutin per sq. cm. of surface.

Discussion.

The work has shown that wide differences exist in the extent of cutin deposition in the cuticles of leaves of different species. Fully grown leaves of apple and black currant show 100 μg of cutin per sq. cm.; others such as tomato, cauliflower and beet show much lower cutin deposits. In contrast to these, tough leaves, e.g. those of *Euonymus*, may contain 500 or more μg of cutin per sq. cm.

The tests with black currant leaves and apple and tomato fruits show that the cutin deposition is most pronounced during periods of rapid growth. Surface area, as well as the cutin deposits per unit area are then increasing, emphasizing the exceptional metabolic activity during these periods. The precise mechanism of cutin deposition in the expanding leaf or fruit has yet to be determined.

The investigations on the phenolic compounds present in apple leaves point to the migration of these substances to the leaf surface. Mann and Wallace in 1924 showed that potassium salts were leached from Cox apple leaves under conditions occurring in the field and concluded that the outer surface layer controlled the loss of water-soluble substances from the leaf (5). Later workers put forward the view that the loss of salts was not a passive leaching effect but the result of an active excretory process in the leaf. Juniper (6) has recently provided evidence, from electron microscope studies of plant surfaces, that there must be a continuous passage of substances through the cuticular layers of leaves, and concludes that the belief that the material external to the epidermal cells is static can no longer be held. Our observations on phenolic and waxy substances on apple leaf surfaces tend to support this contention. The wax and oil fractions in the surface coverings of the apple leaves were reduced towards the end of the season. It may be that, in younger leaves, loss by weathering is matched by the continual formation of waxy material in the leaf cuticle, but that, in old leaves of reduced activity, weathering outpaces replenishment. More definite changes with time were seen in the cuticles of the apple, the oil fraction increasing and the wax fraction diminishing as the fruit ripened. Preliminary trials of the effects of wetting agents upon leaves showed that a solution of sulphosuccinate, at normal strength, applied in large volume to Merton Worcester and Cox apple foliage did not reduce the amount of waxy covering on the leaves.

The work at this stage has been of an exploratory nature, designed to obtain information on the cuticles of plants of economic importance and on the factors influencing cuticle formation. The cuticles of leaves of Merton Worcester and Cox apple trees growing on the same site were very similar,

and no differences were found in the cutin contents of the young and old leaves of Lacatan banana that might account for their different susceptibility to leaf spot disease. The leaves from two strains of cauliflower, var. Cambridge Eight, showed different levels of waxy deposit. Nitrogen deficiency in the apple leaf affected the formation of the waxy covering. Results of the greatest interest have emerged from recent work in this country by Juniper and Bradley (7) and Pfeiffer and his colleagues (8) who have shown that plants grown in soil treated with herbicides such as trichloroacetic acid (TCA) and $\alpha\alpha$ -dichloropropionic acid (dalapon) had greatly diminished waxy deposits and were more susceptible to damage by subsequent herbicidal sprays. In our work, the foliage of strawberry plants that had been damaged earlier by an insecticidal spray showed waxy and cutin deposits lower than those on leaves from normal plants, but whether the reduced cuticle formation was the cause, or the result, of the damage by the spray material is not known. These results show that the chemical treatment of plants may be at least as important as environmental, nutritional or genetical factors in influencing cuticle formation.

Summary.

An account is given of work during 1958 on the cuticles of the leaves of apple, tomato, black currant, strawberry, cauliflower, red beet and banana and of the fruits of apple, tomato and black currant. The changing nature of the cuticle and some of the factors influencing its formation are discussed.

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THE UPTAKE OF FUNGICIDES BY FUNGAL SPORES.

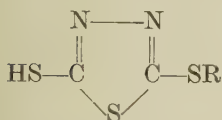
By E. SOMERS.

The toxicity of fungicides on a weight basis (μg . toxicant/g. spore) has been shown (1) to be much lower than that of most animal poisons, insecticides, herbicides, or bactericides. It is also known that many fungicides are non-specific as enzyme inhibitors (2) and that there is little evidence that fungus spores develop a resistance to fungicides (3). All this suggests that fungicides are acting as non-specific, or 'physical,' poisons or else that they are largely detoxified before they reach the active sites on or within the protoplast. In either case the physicochemical properties of the fungicide molecule that

determine its uptake by fungal cells will also determine its toxicity. If the fungicide is to penetrate the cell membrane it will require lipoid solubility (4), for the membrane is considered to be a sandwich of lipoid molecules, such as the glycerides of fatty acids, between two monolayers of protein (5).

A convenient technique for examining the influence of lipoid solubility on the biological properties of a molecule is to study the variation within a homologous series; this technique has not been extensively applied to fungicides. The following is a summarized account of some work on the uptake of a series of mono *S-n*-alkyl dimercaptothiadiazaoles by potato tissue and fungal spores (6).

Thorn and Ludwig (7) prepared 2:5-dimercapto-1:3:4-thiadiazole (DMTD; $R=H$, below) and a series of its *n*-alkyl thioethers, ranging from methyl to octyl; biological tests showed that although the compounds possess appreciable fungicidal activity they are too phytotoxic for field application. The theoretical interest of this series is that structurally they are closely related to ethylene thiuram mono-sulphide, considered by Ludwig, Thorn and Miller (8) to be the degradation product responsible for the fungicidal properties of nabam.



Experimental.

Physical properties of the fungicide series.

DMTD and its *n*-alkyl thioethers (methyl to octyl) were analysed in aqueous solution by their characteristic ultra-violet absorption spectra. The solubility of the crystalline solids in water decreased markedly with increasing length of alkyl side chain up to the amyl homologue. As a measure of the comparative lipoid solubility of the series, mineral oil/water partition coefficients were determined at pHs of 2.0, 5.6, and 7.0, at which the compounds were ionized to the approximate extent of 0.1, 70, and 90%, respectively. The oil/water partition coefficient was found to increase with increasing alkyl side chain up to the amyl homologue and then to decrease for the hexyl, heptyl and octyl members; the effect being most marked at low pH. This is an unusual result for a homologous series and it suggests that there is a tendency to form a lipoid-soluble five-membered ring; this would explain the maximum oil/water partition coefficient and minimum water solubility found with the amyl homologue.

Uptake by potato tissue.

The uptake of fungicides in aqueous solution by disks of conditioned potato tissue has been shown by Ross and Ludwig (9) to correlate well with the uptake of these solutions by fungal spores. When potato disks were immersed in equimolar solutions (1×10^{-4} M) of DMTD and its homologues for a standard period of 2 hr. it was found that the uptake of all the homologues was of the same order with the exception of the amyl member, which gave maximum uptake.

To determine the influence of cell metabolic processes on the uptake by potato tissue, the effects of the following treatments on the uptake of representative homologues were considered: varying fungicide concentration, addition of the salts sodium and potassium chloride, addition of the metabolic

inhibitors potassium cyanide and sodium arsenite, and performing the experiment in an atmosphere of nitrogen. The results of the experiments clearly showed that the uptake by potato tissue was, at least partially, a cell metabolic process. The evidence of some uptake in an atmosphere of nitrogen and in the presence of metabolic inhibitors indicates that part of the uptake process is physical in origin: either simple diffusion into, or adsorption onto, the plant tissue surface, or both processes.

Uptake by fungal spores.

The uptake of the series of fungicides from aqueous solution by conidia of *Aspergillus niger*, *Helminthosporium sativum*, *Monolinia fructicola*, and *Stemphylium sarcinaeforme* was determined from the decrease in fungicide concentration in the supernatant liquid. Uptake by all species was found to be complete within 10 min. Comparison of the uptake by the fungal spores from equimolar concentrations of the homologues showed that uptake increased with increasing length of alkyl side chain to a maximum with the amyl homologue and then decreased for the hexyl, heptyl and octyl members. In experiments with *M. fructicola* it was found that the uptake was doubled when the pH was lowered from 6.0 to 1.5; at this latter pH the compounds are almost completely unionized as compared with 85% ionization at pH 6.

As with potato tissue, a reduction in fungicide uptake in the presence of a metabolic inhibitor or in an atmosphere of nitrogen indicated that the uptake is dependent on cell metabolism although some uptake is probably due to physical processes.

General Conclusions.

The mechanism of toxicity of a fungicide can be considered as due to either a "physical" (structurally non-specific (10)) or a "chemical" action. Ferguson (11) has shown that for a homologous series of physical toxicants there is a direct relation between the logarithms of the equitoxic concentrations and water solubilities. Thorn and Ludwig (7) have determined the toxicity of DMTD and its *n*-alkyl thioethers to *M. fructicola*, but their data, when plotted against solubility, did not conform to Ferguson's Rule. In addition the ratio equitoxic dose/water solubility, used by Ferguson as an approximation to the thermodynamic 'activity' of the toxicant, was below the value (0.1) considered by Ferguson to show physical toxicity. Thus it appears that the toxicity of DMTD and its homologues is due to a chemical mechanism determined by the number of molecules reaching the site of toxic action in the fungal spore.

Fig. 1 shows the relationship between the oil/water partition coefficient, equitoxic concentration and uptake by *M. fructicola* for members of the homologous series. The marked parallelism between the toxicity, uptake, and partition coefficients of the compounds suggests that their toxicity is related to their penetration of the cytoplasmic membrane. The most toxic homologues, butyl and amyl, have also maximum uptake and oil/water partition coefficients.

Calculation of the uptake of the amyl homologue gave values ranging from 67,000 for *A. niger* to 4,400 for *M. fructicola*, expressed as $\mu\text{g. fungicide/g. spore}$. These very high values are similar to those obtained by other workers (1, 12) and indicate that as the fungicides are not acting as physical poisons the spores are probably detoxifying an appreciable proportion of the fungicides

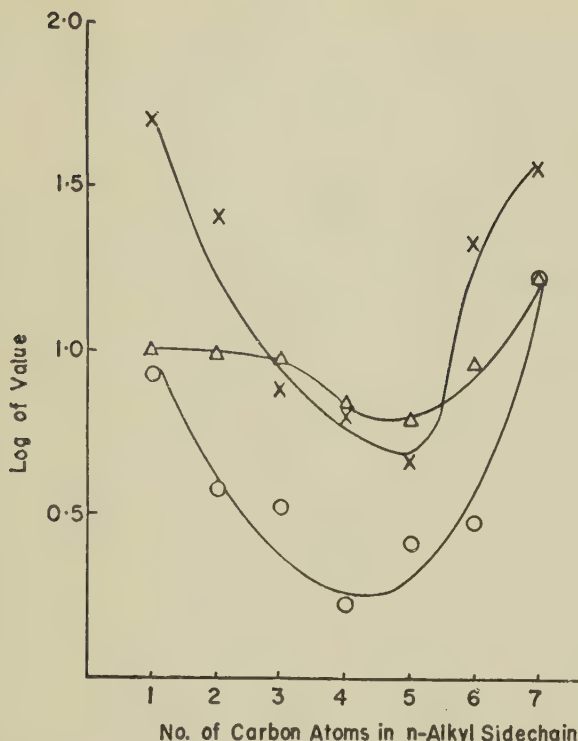


Fig. 1. Properties of the DMTD homologues

○ = ED₅₀ *M. fruticola* (moles $\times 10^{-6}$ /litre) (from Thorn and Ludwig (7)).

× = $10 \div$ Partition coefficient.

Δ = $100 \div$ Uptake by *M. fruticola* (moles $\times 10^{-9}$ /million spores).

either in the cell wall or within the protoplasm. The resultant toxicity of DMTD and its homologues is probably due to chemical interaction with a lipophilic biophase of the fungal cell; the greatest toxicity would then be associated with maximum access to this biophase. The hypotheses suggested above are now being applied to studies of the toxicity of some analogues of captan, a fungicide considered (13) to be non-specific in its action, and of the toxicity of cupric ion, an enzyme inhibitor and chemical poison.

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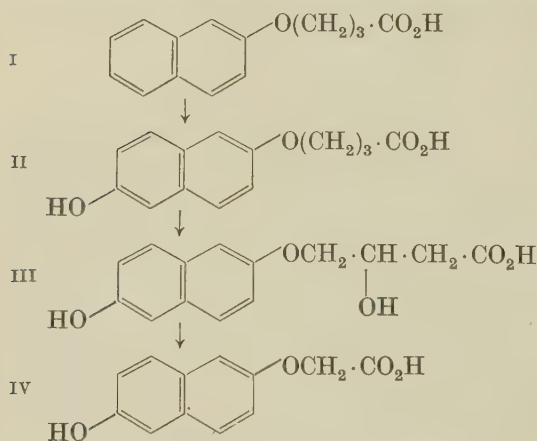
THE METABOLISM OF AROMATIC COMPOUNDS BY FUNGI.

By D. WOODCOCK and R. J. W. BYRDE.

The importance of detoxication in many biological systems is becoming increasingly apparent, but the metabolism of toxic materials by fungi has, apart from a few specific instances, received little attention. The fundamental importance of detoxication in fungicidal action led to the commencement of systematic studies at Long Ashton in 1954; some results of this work are outlined here.

*Metabolism of ω -(2-naphthylloxy)-*n*-alkylcarboxylic acids.*

Whilst there is considerable evidence, both direct and indirect, that acids of this type undergo β -oxidation in plants, little was known in 1954 of their metabolism by micro-organisms, though Rittenberg & Ivler (1) had shown that phenyl-substituted fatty acids are oxidised by bacteria, and Webley, Duff & Farmer (2) have since studied their breakdown by *Nocardia opaca*. Using a replacement culture technique with *Aspergillus niger* van Tiegh (Mulder strain) it has been found that ω -(2-naphthylloxy)-*n*-alkyl-carboxylic acids undergo nuclear hydroxylation as well as β -oxidation of the side-chain (3). Thus γ -(2-naphthylloxy)-*n*-butyric acid (I) is converted to 6-hydroxy-2-naphthylloxyacetic acid (IV) by the metabolic pathway shown, and chromatographic examination of the substrate has clearly shown that nuclear



hydroxylation to (6-hydroxy-2-naphthylloxy)-*n*-butyric acid (II) is rapid and thus takes precedence over β -oxidation. The presence of β -hydroxy- γ -(6-hydroxy-2-naphthylloxy)-*n*-butyric acid (III) as an intermediate has been established with reasonable certainty by a comparison of the ultraviolet absorption spectra of the isolated metabolite and a synthetic acid, though owing to the lability of this compound neither sample has yet been obtained analytically pure (4).

Although acids having an even number of carbon atoms in the side-chain were successively β -oxidised to 6-hydroxy-2-naphthylloxyacetic acid, the corresponding acids with an odd number of carbon atoms provide an anomaly. There is so far no evidence that these acids are degraded further than β -(6-hydroxy-2-naphthylloxy)-propionic acid by *A. niger*; the reason for the failure of β -oxidation at this stage is still not clear.

Using *Sclerotinia laxa* Aderh. & Ruhl., naphthylloxy acids were found to undergo β -oxidation with some reluctance, but degradation of the propionic acid side-chain did in fact take place with this fungus, β -naphthol being isolated from acids with an odd number of side-chain carbon atoms. *S. laxa*

was, however, a complete contrast to *A. niger*, since nuclear hydroxylation was virtually absent. *Botrytis cinerea* was also incapable of the nuclear hydroxylation of these naphthylxy acids and it is not at all certain whether this fungus can even carry out β -oxidation.

Metabolism of phenoxy-n-alkylcarboxylic acids.

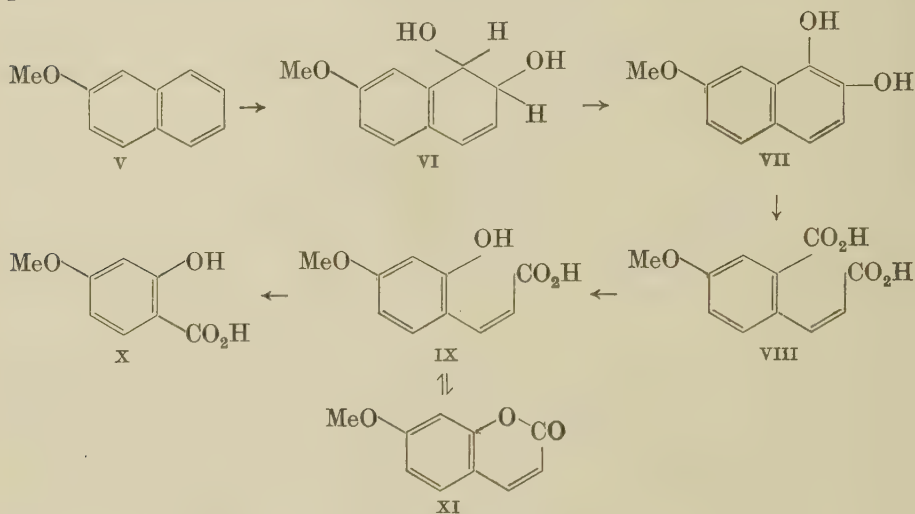
The results obtained with these acids (5) followed the general pattern of those in the 2-naphthylxy series. β -oxidation of the side-chain once again took place and resistance of the propionic acid side-chain to further oxidation was also noted. Whereas hydroxylation was limited to the 6-position in the naphthalene nucleus, it occurred in both the *para*- and to a lesser extent in the *ortho*-position of the phenoxy acids. By contrast, hydroxylation of phenoxyacetic acid using the Fe^{++} -ascorbic acid model system of Udenfriend, Clark, Axelrod & Brodie (6) took place primarily in the *ortho*-position, though *para*-substitution did occur to some extent.

Metabolism of 2-methoxynaphthalene.

In the work so far described on the metabolism of ω -(2-naphthylxy)-*n*-alkylcarboxylic acids by *A. niger* and *S. laxa*, there was little evidence of ring fission by either fungus, which was in marked contrast to the degradation of naphthalene and some substituted naphthalene compounds by soil microorganisms (7, 8, 9). In order to examine the significance of a non-polar sole-carbon source in ring fission by fungi, 2-methoxynaphthalene was used in the replacement culture technique with *A. niger* (10). The major metabolic product isolated was shown to be 4-methoxysalicylic acid, and the analogy between this compound and 3-chlorosalicylic acid, which was obtained by Walker & Wiltshire (9) from 1-chloronaphthalene using soil bacteria, suggests a similar metabolic pathway. However, although these workers were able to isolate D-8-chloro-1:2-dihydro-1:2-dihydroxynaphthalene from 1-chloronaphthalene and regarded it as a precursor of 3-chlorosalicylic acid, we were unable to isolate any similar diol from 2-methoxynaphthalene. Moreover, 5-, 6-, 7- or 8-hydroxy-2-methoxynaphthalene, which would be formed by acid treatment of such a diol, could not be detected chromatographically. Other failures to isolate diol intermediates have been reported by Canonica, Fiecchi and Treccani (11) who employed *Pseudomonas desmolytica* for the metabolism of 1-methylnaphthalene and by Murphy & Stone (12), who failed to isolate D-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene from a *Pseudomonas* strain growing on naphthalene.

Considering the oxidative breakdown of 2-methoxynaphthalene, the presence of the hydroxyl group in the final product suggests that this substitution must occur after the initial ring fission, otherwise the alkoxyl-substituted ring would have been more active originally and hence degraded with consequent loss of the alkoxyl group. This possibility is regarded as improbable since the formation of 4-ethoxysalicylic acid from 2-ethoxynaphthalene has now been confirmed. Hydroxylation following ring-fission is a feature of the scheme put forward by Fernley & Evans (13) for the end-ring attack on naphthalene by soil *Pseudomonads*. A similar route would seem to be most likely for the formation of 4-methoxysalicylic acid (X) from 2-methoxynaphthalene (V), the metabolic pathway being by way of D-*trans*-1:2-dihydro-1:2-dihydroxy-7-methoxynaphthalene (VI), 1:2-dihydroxy-7-methoxynaphthalene

(VII), 2-carboxy-4-methoxy-*cis*-cinnamic acid (VIII) and 2-hydroxy-4-methoxy-*cis*-cinnamic acid (IX). The failure of *A. niger* to metabolize 2-carboxy-5-methoxycinnamic acid to 4-methoxysalicylic acid may be explained by stereospecificity of the fungal enzyme (cf. Evans & Smith (14)). Chromatographic indications of the presence of 7-methoxy-coumarin (XI) support the postulated route via 2-hydroxy-4-methoxy-*cis*-cinnamic acid.



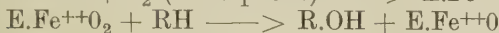
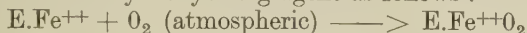
Metabolism of 3-indolylacetic acid.

Work on the metabolism of this compound by *A. niger* is still in its early stages. Preliminary indications are that hydroxylation takes place in both 5- and 7-positions, but that it is accompanied by extensive ring fission (15). The presence of an aldehydic breakdown product has been demonstrated chromatographically but there have so far been no indications of the presence of salicylic acid, recently reported by Proctor (16) as a product of the metabolism of 3-indolylacetic acid by means of an unidentified *Pseudomonas*.

Mechanism of hydroxylation.

Hydroxylation is a common primary metabolic consequence when animals or micro-organisms are subjected to the action of foreign aromatic substances. The significance of the formation of dihydrodiols by the action of micro-organisms on compounds containing double bonds is at present undecided. Mitoma and his colleagues (17) have shown that the enzymic intermediate involved in perhydroxylation is almost certainly a positively charged ferro-protein complex containing oxygen derived from the atmosphere. If the enzyme-substrate complex is assumed to be an oxonium ion of the epoxide type, formed by attack upon a double bond by the enzyme-oxygen complex, nucleophilic attack by 2 OH^- would lead to the formation of a *trans*-diol, and in fact the only diols so far isolated possess *trans*- and not *cis*-configurations. Where diols have not been isolated it seems reasonable to assume that utilization takes place too rapidly for detection (10, 11, 12). Walker & Wiltshire (9) consider that such diols are true metabolic intermediates, whereas phenols or naphthols may be artefacts or may arise from side-reactions.

Some light has been thrown on non-specific hydroxylation by the study of model (i.e. non-enzymic) hydroxylating systems. Using the Fe^{++} -ascorbic acid system devised by Udenfriend and his co-workers (6), the products obtained from a number of aromatic compounds are similar to those obtained from animal studies. At Long Ashton this model system gave results with aryloxyalkyl-carboxylic acids in good agreement with those obtained using *A. niger* (3, 5). The actual oxidizing species in the model system is not known, though hydroxylation appears to occur only at electronegative sites in aromatic and heterocyclic ring systems, as indeed happens with the fungus. This would suggest that hydroxylation is carried out by a cationic fragment such as OH^+ , which might arise by the action of ionising radiation on water or from decomposition of peracids. Alternatively some enzyme-iron-oxygen complex may act as a hydroxylating agent as follows :



Dalglish (18) has, however, pointed out that a free-radical electron acceptor of the dihydroascorbic acid type may itself be the active oxidizing agent. This apparently anomalous attack at electronegative sites has recently been shown by Dewar (19), using the molecular orbital approach, to be compatible with a free radical mechanism.

It seems likely that hydroxylation by *A. niger* is associated with sporulation and melanin formation, since no hydroxy-acids could be detected when the fungus was grown in submerged cultures, in which it failed to sporulate or pigment. Whether trace-metals play a significant part is as yet uncertain ; in preliminary experiments the addition of sodium diethyldithiocarbamate markedly inhibited hydroxylation.

The introduction of the phenolic -OH group, long regarded as a potential toxophore from the fungal standpoint, is of great biological as well as chemical interest. Thus 4-ethoxysalicylic acid, produced in substantial amount from 2-ethoxynaphthalene by *A. niger*, provides an example of a breakdown product more fungitoxic than the initial substrate.

Such facile entry of a hydroxyl group promises useful application in synthesis ; model systems such as Udenfriend's may prove capable of introducing such groups, under the mildest of conditions, at selected sites in aromatic rings.

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SPRAYING EXPERIMENTS AGAINST APPLE SCAB AT LONG ASHTON, 1958.

By R. J. W. BYRDE, G. M. CLARKE, C. W. HARPER and NORA M. WAUGH.

Preliminary laboratory tests of some potential orchard fungicides indicated that *n*-dodecylguanidine acetate (Cyprex), 2:4-dichloro-6-(*o*-chloro-anilino)-*s*-triazine and *o*-hydroxy-diphenyl showed sufficient promise to warrant trial in the field against apple scab, caused by *Venturia inaequalis* (Cooke) Wint. (1). These three compounds, together with a new organo-mercury fungicide, were therefore included in 1958 in a pilot-scale trial on Worcester Pearmain apples which forms the subject of this paper.

Experimental.

Fungicides. The spray materials used were as follows :—

o-Hydroxy-diphenyl, as a 50% wettable powder on a kaolin base, dispersed with the aid of sodium carboxymethyl cellulose (final cellulose concentration in spray, 0.02%).

2:4-Dichloro-6-(*o*-chloroanilino)-*s*-triazine, similarly formulated.

n-Dodecylguanidine acetate, as a commercial wettable powder containing 65% active material.

Phenyl mercury salicylanilide, as a commercial sample of a miscible liquid containing 0.4 lb. mercury per gal.

Captan, as a commercial wettable powder containing 50% active material.

Rates of application are listed in Table I. The sprays were applied by means of a mobile machine using a triple-nozzle broom with 4/64 in. disc apertures and one short-range and two long-range swirl plates. The output was approximately 3½ gal. per min. Dates of application were : 10th May, 5th June, 26th June.

Trees. The plot used comprised twenty-nine 23-year-old bush trees of the variety Worcester Pearmain, standing in grass at 21 ft. apart. They had received tar-oil winter wash early in the year, but no further insecticide or acaricide was applied until after picking, to avoid any chance of interaction with the experimental fungicides.

Layout. Each of the six treatments was replicated five times in a Youden Square (a 6×6 Latin Square with one row omitted), so that the analysis eliminated two orthogonal sources of variation (N.-S. and E.-W.) and the single missing tree could easily be allowed for. The layout was such that residual effects of these treatments will be capable of examination in 1959 when a different set of five treatments can also be tested.

Methods of Assessment. Mean percentage scab areas per leaf and per fruit were estimated by Tehon and Stout's method, as amplified by Marsh (2), and were based on samples of 250 leaves and 50 fruits.

An approximate estimate of red spider damage was made by classifying each tree into one of five categories—0 (none) to 4 (severe).

Notes on weather. The summer of 1958 was predominantly wet and sunless. The rainfall figures (in.) for each month are shown below with average values (1914-53) in brackets :—

April, 1.25 (2.33) ; May, 3.58 (2.59) ; June, 3.63 (2.11) ; July, 1.99 (3.16) ; August, 3.07 (3.65) ; September, 5.47 (3.18).

Total 18.99 (16.99). Excess over normal 11.8%.

Results.

Apple scab was estimated on leaf samples on July 21st–25th and on fruit samples on September 4th–5th. Results are shown in Table I.

TABLE I.
MEAN PERCENTAGE LEAF AND FRUIT SCAB ON SAMPLES.

Fungicide	Concn. active material (lb./100 gal.)	% Scab	
		Leaf	Fruit
<i>n</i> -Dodecylguanidine acetate	0.91†	0.68	0.18
Phenyl mercury salicylanilide	0.02 (as Hg)	0.71	0.38
2:4-Dichloro-6-(<i>o</i> -chloroanilino)- <i>s</i> -triazine	1.0	1.07	0.45
Captan	1.0	1.05	0.87
<i>o</i> -Hydroxy-diphenyl	1.0	2.39	1.48
None (Control)	—	4.15	1.38
Least significant difference (5% level) ..		0.97	0.61

† 1.27 lb./100 gal. applied in error on second spraying date.

In the course of the fruit assessment, the prevalence of disfiguring russet was recorded. The percentage of fruits affected was virtually nil for all treatments except the *s*-triazine compound, for which the figure was almost 100%.

Red spider damage on the trees was assessed on July 21st, when the following mean indices of damage were recorded :— phenyl mercury salicylanilide, 1.75 ; *n*-dodecylguanidine acetate, 1.89 ; captan, 2.24 ; *o*-hydroxy-diphenyl, 2.52 ; 2:4-dichloro-6-(*o*-chloroanilino)-*s*-triazine, 3.09 ; control, 3.71. Least significant difference (5% level) : 0.62.

There was a slight incidence of apple mildew (caused by *Podosphaera leucotricha*) in the course of the trial, although the variety Worcester Pearmain is normally resistant to this disease. Preliminary counts indicated that the *n*-dodecylguanidine acetate and phenyl mercury salicylanilide treatments might be having some checking effect on the disease. The incidence of the disease was too low for reliable data to be obtained for statistical analysis.

Discussion.

Although the scab inoculum at the beginning of the 1958 season was probably small, the weather conditions at Long Ashton in the summer of 1958 were favourable to the development and spread of the disease. The fungicidal treatments described above, applied only three times during the season, thus received a stringent test. Captan, for example, which was included as a standard, failed to reduce scab to a level which would be commercially acceptable, though previous experience (3) has shown that a full spray programme gives adequate scab control.

The new fungicide *n*-dodecylguanidine acetate ($C_{12}H_{25}-NH-C(=NH)-NH_2$, CH_3COOH) showed great promise as a scab fungicide. This compound, which had not been tested in England until 1958, had already proved itself equal to or better than captan in North America. Thus Powell, Khettry, Sasaki & Brussell (4) found that a 70% preparation used at $\frac{3}{4}$, 1 or $1\frac{1}{2}$ lb. per 100 gal.(U.S.) gave 99% control of scab. Its ability to protect foliage increased with the frequency of spraying, but as few as 3 sprays gave excellent control of fruit infection. This finding—with which the Long Ashton results are in agreement—was thought to be due to the great persistence of small quantities of spray residue. Hamilton & Szkolnik (5) also found the material very effective against scab, and showed that some local systemic effect was exerted by the compound: the top surface of a leaf was protected when only the under-surface was sprayed. Cation (6) found *n*-dodecylguanidine acetate better than captan for scab control, but liable to cause fruit russet. This russetting may be associated with application coinciding with night frosts.

The organo-mercurial compound, phenyl mercury salicylanilide, also brought about a marked reduction of apple scab. It should be emphasized that, since the spray applications were not deliberately timed in relation to infection periods (which would have been impossible with only three applications), this compound may have been at some disadvantage.

The *s*-triazine compound and *o*-hydroxy-diphenyl were clearly inferior: the former compound caused severe russetting on Worcester, not normally an unduly sensitive variety, whilst the latter had little effect on scab.

Further trials of *n*-dodecylguanidine acetate are planned: information is particularly required on its effect on apple mildew on a susceptible variety, and its phytotoxicity on Cox's Orange Pippin. A single tree of Cox which received a single spray in June 1958 showed no obvious damage, but two or three seasons' experience on a range of varieties will be needed before the material can be recommended for large-scale use.

Summary.

Five fungicides, each applied three times during the season, were tested in a small-scale trial against apple scab (caused by *Venturia inaequalis*) on Worcester Pearmain bush apple trees.

n-Dodecylguanidine acetate (0.091%) and phenyl mercury salicylanilide (0.002% Hg) gave promising results, reducing leaf scab by 84% and 83%, and fruit scab by 87% and 72% respectively. No phytotoxicity was observed. Both compounds appeared to check a light attack of apple mildew (caused by *Podosphaera leucotricha*) and also to reduce damage by red spider mite.

o-Hydroxy-diphenyl and 2:4-dichloro-6-(*o*-chloroanilino)-*s*-triazine were unsatisfactory: the former had little effect on scab whilst the latter caused severe fruit russet.

Acknowledgments.

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THE EFFECT OF COVER CROPS ON THE SUSCEPTIBILITY OF COX'S ORANGE PIPPIN APPLES TO INFECTION WITH *GLOEOSPORIUM PERENNANS*.

By A. T. K. CORKE.

Bould and Jarrett (1) have described the initial changes in the growth and leaf nutrient status of three-year-old Cox and Worcester trees, after cover crops were sown on an experimental plot at Long Ashton in April, 1955: the cover crops were white clover, timothy grass, perennial rye grass and "tumble down" (natural sward).

On the four replicates of each cover crop are superimposed two replicates of eight fertilizer treatments (NPK factorial): a further single replicate is applied to an adjacent arable plot. The whole cover crop area receives a dressing of Nitro-chalk at 2 cwt. per acre each spring (basic nitrogen level); half of the 64 sub-plots receive an additional 2 cwt. per acre of Nitro-chalk as part of the fertilizer treatment (additional level). Each sub-plot consists of two recorded trees, one Cox and one Worcester. No over-all spring dressing of Nitro-chalk is given to the arable plot, and to the four sub-plots receiving nitrogen in the fertilizer treatment it is applied at 1 cwt. per acre.

Nitrogen deficiency, which was apparent from the leaves of all the trees under cover crops in June, 1955, became less severe in 1956 on the clover and "tumble down" plots, but more severe on the two grass plots. Compared with that of trees on the arable plot, the leaf nitrogen status was then almost normal in trees under clover, but was slightly deficient in trees on "tumble down" plots and severely deficient in trees on timothy and perennial rye grass plots. Additional nitrogen had a significant positive effect on the leaf nitrogen status of the Cox trees (1).

In 1957 and 1958 fruits harvested from each one of the Cox's Orange Pippin trees were inoculated with *Gloeosporium perennans*. The rotting which resulted was examined in relation to the cover crop and the level of nitrogen applied to the trees which bore the fruits.

Experimental.

1957. The first crop picked from the trees growing under cover crops was harvested on 18th–19th September, 1957. Where possible, five apples were taken from each tree (Cox), to give ten fruits per treatment (Cover $\times$ Fertilizer). Crops were very small on trees under the grass covers, trees on half the plots yielding less than ten apples, and no fruit at all on two plots under perennial rye. The apples were kept at 10°C. for one day; eight apples from each treatment were then inoculated with *Gloeosporium perennans* by immersion in a suspension of conidia (50,000 per ml.) for 10 seconds; the remainder, approximately two apples per treatment, were dipped in water to provide controls. After 10 minutes the fruits were placed in trays under a plastic cover, and kept for 3 days at room temperature, before being stored at 3°C.

1958. The fruit was gathered on 25th–26th September and placed in store at 3°C. for 4 days before inoculation. On this occasion, a sample of twenty apples from the trees receiving basic nitrogen, and twenty from the trees receiving additional nitrogen were taken from the clover and “tumble-down” plots; ten apples in each of these categories were taken from the trees on the timothy and perennial rye grass plots. The apples were sprayed with a suspension of conidia (100,000 per ml.); after 5 minutes they were turned over and sprayed again. After being placed in trays, the fruits were kept under a plastic cover for 7 days at room temperature, before being stored at 3°C. Two apples per treatment were sprayed with water to provide controls.

An assessment of the surface area (%) occupied by the rots was made after one month, and then at fortnightly intervals until late February.

Results.

The results obtained from the 1957 crop are shown in Fig. 1.

Following inoculation, the rotting of fruits from trees growing under clover was much the most rapid and, while there were some differences between the rates of rotting of fruits from trees growing under the other covers up to 130 days after inoculation (28th January, 1958), these were small. The “control” values are those obtained from uninoculated fruits picked from the same trees.

Inoculation of the 1958 crop again showed that rotting was fastest on fruits from the clover plots (Fig. 2) but, in this case, there was a more obvious suggestion at first of a correlation between the rates of rotting and the nitrogen levels in the trees on the different plots.

The 1958 crop was picked a week later, and inoculated 12 days later than the 1957 crop but, because of the higher rate of rotting, the mean area rotted had exceeded that of the 1957 crop by early January (103 days after inoculation).

Examination of the rotting of fruits from trees receiving different fertilizer treatments, irrespective of the cover crops, indicated that rotting was not always more rapid on fruits from trees given extra nitrogen. Although the number of apples was greater on trees receiving extra nitrogen under all the covers, the increase was least marked in 1957 on the perennial rye plots, and in 1958 on the timothy plots. Again, increases in the mean weight of the fruits occurred only on the trees receiving additional nitrogen under perennial

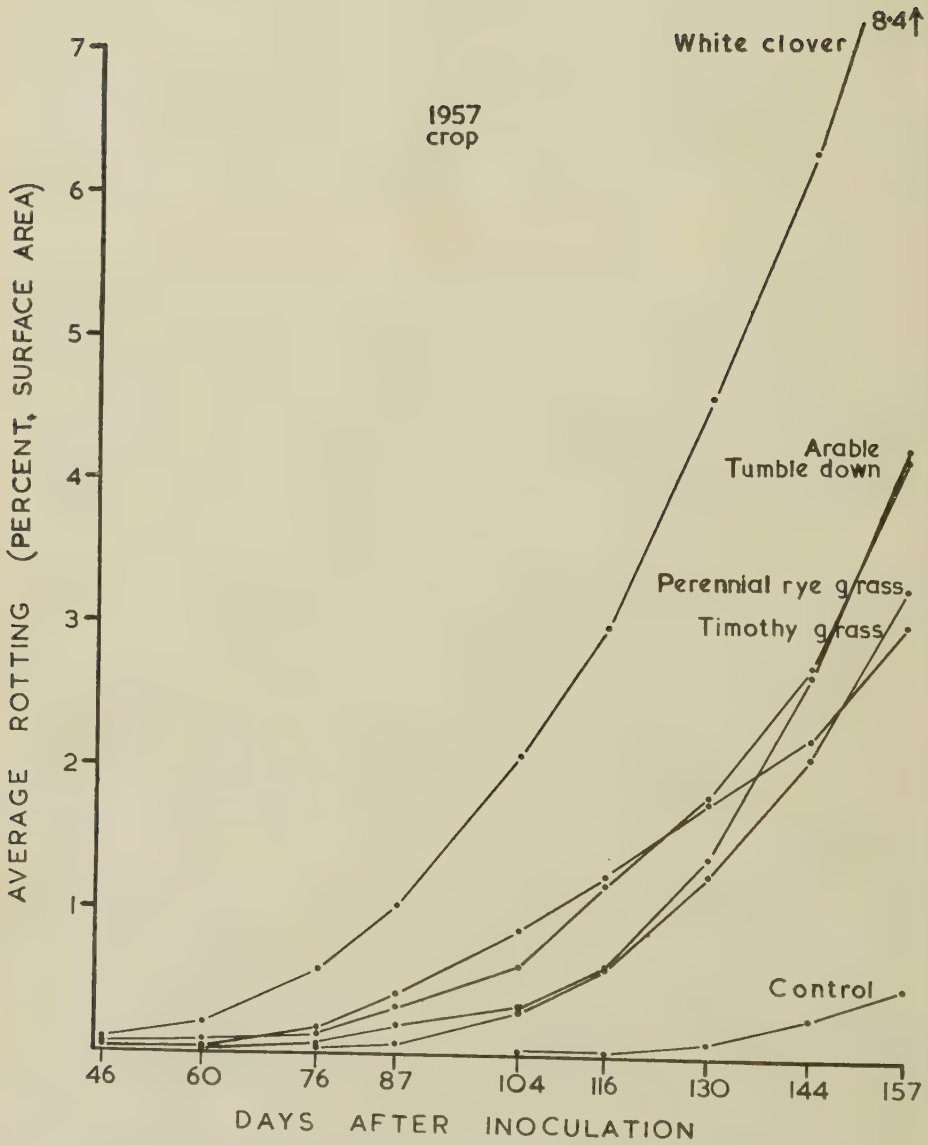


Fig. 1. Average rotting of inoculated and uninoculated fruits from 1957 crop.

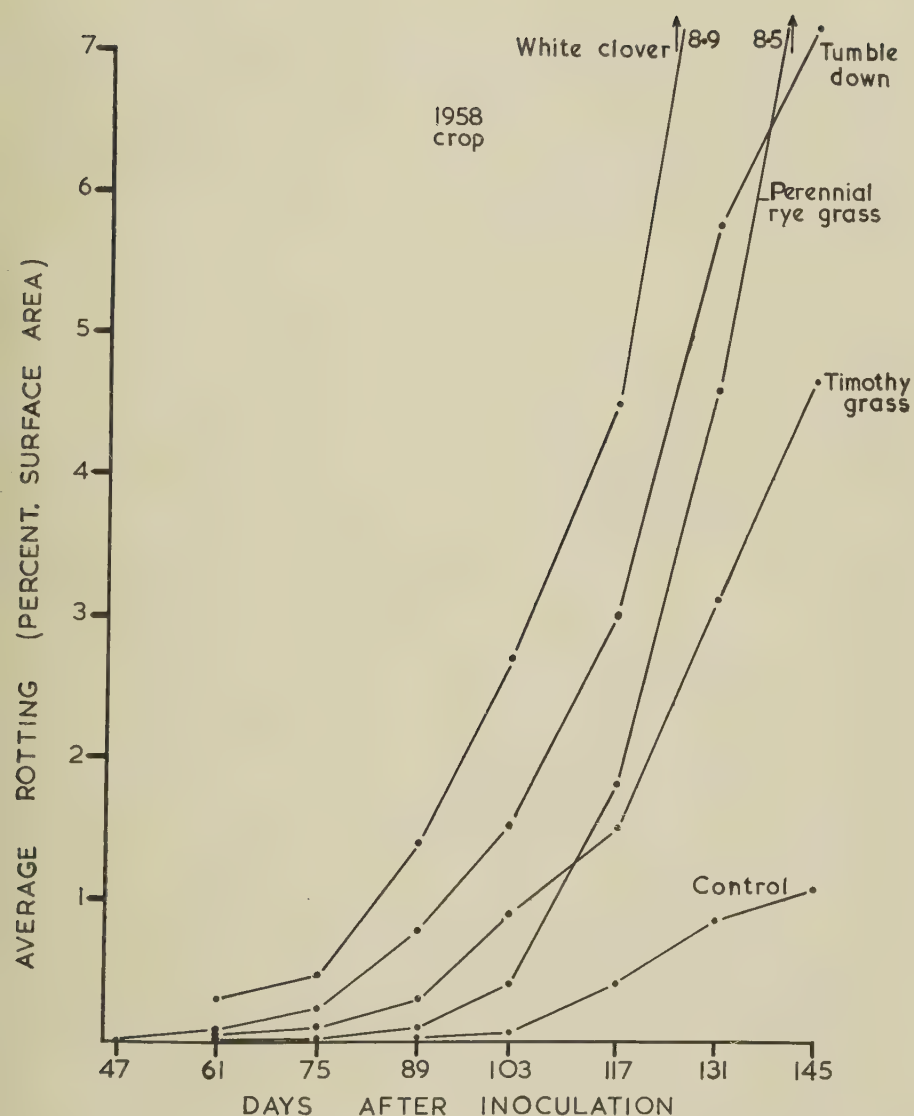


Fig. 2. Average rotting of inoculated and uninoculated fruits from 1958 crop.

rye and timothy in 1957, and under timothy in 1958. In 1957, except for fruits from trees under perennial rye, the rate of rotting was much faster on fruits from trees given additional nitrogen: fruits from perennial rye plots rotted at almost the same rate whether extra nitrogen had been given to the trees or not. In 1958, however, fruits from trees growing under perennial rye and timothy, and given extra nitrogen, rotted much more slowly than those from trees given the basic level of nitrogen; the rotting of fruits from the clover and "tumble down" plots was much the same as in 1957.

It is worthy of note that in 1957 the rate of rotting of fruits from trees on the arable plot receiving no nitrogen was twice that of fruits from trees on this plot given nitrogen in the fertilizer treatments (1 cwt. Nitro-chalk per acre).

The rate of rotting of fruits from trees under the covers receiving additional nitrogen (4 cwt. Nitro-chalk per acre) is compared in Fig. 3 with that of fruits from trees receiving the basic level (2 cwt. per acre). The difference in behaviour, in the two years, of fruits from trees on grass plots receiving the two levels of nitrogen, is reflected in the steep rise of both lines in 1958.

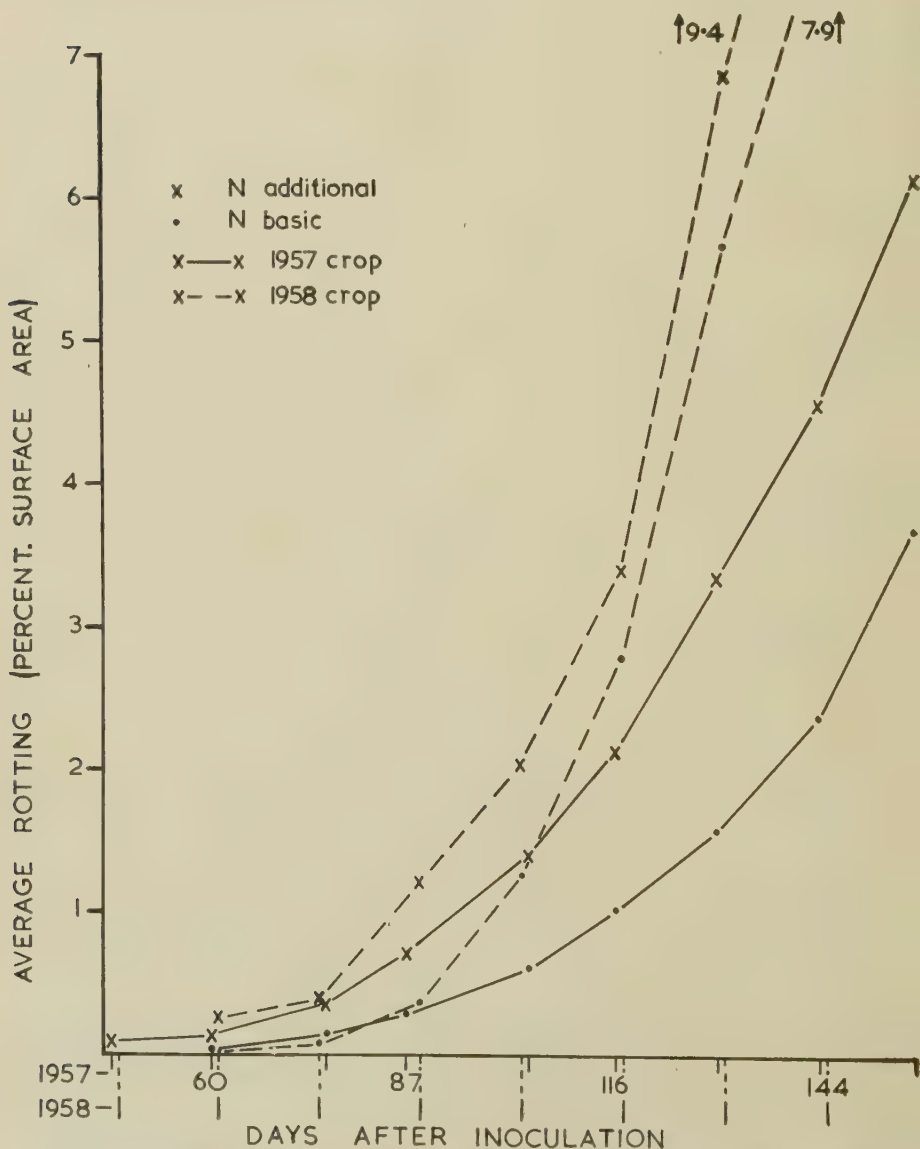


Fig. 3. Average rotting of inoculated fruits from trees receiving basic or additional nitrogen, 1957 and 1958.

Discussion.

During spray trials against orchard infections of Allington Pippin apples by *Gloeosporium perennans*, Marsh and Edney (6) noted a reduction in susceptibility following the grassing-down of the orchard, a process which reduces the level of nitrogen in the trees. The experiment described above suggests that the degree of susceptibility of Cox's Orange Pippin fruits to *Gloeosporium* infection is correlated with the level of nitrogen in the tree. The factors affecting the susceptibility of fruits on large, established trees may differ from those in young trees: the small leaf area on young trees deficient in nitrogen, for example, may influence fruit development to a degree not detectable in large trees.

Edney (3) has found that susceptibility to *Gloeosporium* infection increases as the fruit develops on the tree, and that small fruits are less susceptible at harvest than large. In general, small fruits are more coloured than larger fruits, and there is evidence that the observed reduction in the rate of rotting of some fungi on the blushed side of an apple is due to tissue firmness (5). A species of fungus that extends mainly by intercellular penetration, and produces little enzyme, rots the softer, green side more rapidly than the red. On the other hand, one that extends by killing the cells of the apple in advance of the hyphae, by producing toxic substances, shows no such preference. *G. perennans* is of the former type (3).

In this experiment an increased susceptibility to rotting was recorded in 1957 and 1958 on fruits from trees receiving additional nitrogen under clover and "tumble down," although there was no difference between the mean weight of these fruits and of those from trees given no extra nitrogen. However, this was not true of fruits from trees under perennial rye or timothy. Here it was found that in 1957 the mean weight of the fruits from trees under timothy and perennial rye, given additional nitrogen, was greater than that of fruits from trees given the basic level: rotting was faster on the heavier fruits from the timothy plots, but almost the same on fruits from all the plots under perennial rye. In 1958, heavier fruits were recorded only from trees under timothy given extra nitrogen, but on this occasion the rate of rotting of fruits from trees given additional nitrogen on both perennial rye and timothy plots was slower than on fruits from trees given the basic level of nitrogen.

These results suggest that apples may be more susceptible to rotting by this fungus if the level of nitrogen, in the tree on which they are borne, departs from the normal towards excess or deficiency. If this were so, the rise in the level of leaf nitrogen recorded in 1956 on trees under clover and "tumble down" would have resulted in an increased susceptibility to rotting, made even greater by the application of additional nitrogen. On the other hand, increasing deficiency of nitrogen would also have increased the susceptibility of the fruits to rotting, but the effect would have been made less by the application of extra nitrogen. In both 1957 and 1958 fruits from the "tumble down" and timothy plots showed an intermediate rate of rotting, suggesting that the level of nitrogen in the trees on these plots is not far removed from that at which the fruit would be least susceptible to rotting by *G. perennans*.

In view of the incomplete range of samples of fruits from the perennial rye and timothy plots in 1957, and the results obtained with fruits from the arable plots, further results will have to be available before this hypothesis can be examined more fully.

It has been found in some instances that susceptibility to disease is increased when the level of carbohydrates within the plant is raised (4, 7); there is also evidence that the susceptibility of Cox's apples to *Gloeosporium* infection is decreased, at least for a time, by reducing the leaf area of the trees (2). Such an effect has been observed after summer pruning, which results in the removal of a very small proportion of the leaves on the trees. However, the developing fruits are largely dependent on leaf activity for the supply of carbohydrates, so that small variations may be sufficient to produce a measurable change in the susceptibility of fruits to rotting.

Edney has identified two factors concerned in the resistance of an apple to infection by *G. perennans*: the suberization of cells below the epidermis, and the resistance of the cells of the cortex. It seems possible that part, at least, of the resistance of the cortex may depend on the constitution of the cell sap. With small trees, such as those concerned in this experiment, the effects of nitrogen deficiency are so clearly seen as to suggest that in the supply of carbohydrates may lie the only source of variation in susceptibility to rotting. However, there is possibly a more direct correlation between the rate of rotting and the level of nitrogen in the tree.

Summary.

Records of the rotting of Cox's Orange Pippin fruits, picked from trees in a cover crop/manurial experiment and inoculated with *Gloeosporium perennans*, suggested a correlation between the rate of rotting and the level of nitrogen in the tree. In fruits of the same mean weight from the same cover treatments, a higher rate of rotting was recorded on fruits from trees that had received additional nitrogen. In 1958, however, fruits from trees under perennial rye and timothy given additional nitrogen rotted more slowly than those from corresponding trees given the basic level of nitrogen.

Acknowledgments.

The author is indebted to Dr. C. Bould and Mr. R. M. Jarrett for access to the records of treatments and crop weights; also to Miss M. Marlow for mycological assistance.

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COMPARATIVE OBSERVATIONS ON APPLE SHOOT INFECTIONS OF *GLOEOSPORIUM PERENNANS* AND *NECTRIA GALLIGENA*.

By R. O. SHARPLES.*

Introduction.

Since the conidial and ascospore stages of *Gloeosporium perennans* and *Nectria galligena* are readily distinguished, fruiting cankers caused by these two fungi are easily identified. When, however, no fructifications are being produced, cankers caused by *G. perennans* may be similar in external appearance to the common canker of apple trees caused by *N. galligena*. As the type of economic damage and the methods of control of these two fungal diseases differ in certain respects, a series of comparative observations was carried out to establish possible criteria for distinguishing between lesions due to *N. galligena* and those due to *G. perennans*.

External Characters.

Lesions of *G. perennans* may arise naturally as dieback infections originating in a distal pruning wound or as cankers originating in lateral pruning cuts or wounds. The lateral infections, which extend particularly rapidly when initiated during the dormant season, form elongate, dark coloured flattened lesions. The periderm splits into longitudinal strips which peel away from the swollen cortex. (Plate XIII, Fig. 1). Similarly, infections of *N. galligena* (apart from those arising at leaf scars) may originate from pruning or other wounds. On older branches, however, *Nectria* cankers may also develop around the base of an infected lateral; or latent infections, present in partially healed pruning wounds, may renew their growth. During the early stages of development the *Nectria* lesions closely resemble those caused by *G. perennans* but as the bark begins to dry out it breaks up into numerous small rough scales. The ragged, squamous appearance is particularly obvious in cankers on branches more than one year old and is distinct from the extensive papery peeling of periderm that is characteristic of *Gloeosporium* infections (Plate XIII, Fig. 1). Typical lesions of *N. galligena*, viewed from the side, show a concavity around the point of infection, in contrast with the level, parallel-sided appearance of a shoot infected by *G. perennans* (Plate XIII, Fig. 1).

Infections of *N. galligena* on one-year-old shoots frequently originate at a leaf scar (1) and, although similar outbreaks of leaf-scar infections of *G. perennans* have not been recorded in commercial orchards, the development of lesions of this type has recently been demonstrated by artificial inoculation experiments (2). Similar lesions were also found to originate naturally in leaf scars situated approximately 5 cm. below short snag infections of *G. perennans*. The leaf scar infections, which were not apparent until the following spring, developed into dark-brown, flattened lesions from which the periderm peeled away in relatively large fragments. Although in their early development these lesions resembled leaf scar infections of *N. galligena*, by mid-June a conspicuous callus ridge, characteristic of *G. perennans* infections, surrounded the diseased tissues: by then the lesions were also producing acervuli.

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Anatomical Details.

Distribution of mycelium.

The anatomical development of shoot infections of *N. galligena* and *G. perennans*, on bush apple trees of the variety Cox's Orange Pippin, has been described in detail by Crowdy (3) and Sharples (4) respectively. The general distribution of mycelium at an early stage in the development of *N. galligena* lesions is essentially similar to that in cankers of *G. perennans* of a comparable age, with intracellular hyphae growing through the phloem sieve tubes, xylem vessels and fibres, and intercellular mycelium spreading less rapidly in the parenchymatous peripheral tissues. In both types of lesions the hyphae invade the individual cortical cells only at a later stage. It appears, from sections of comparable infections of both types collected during spring, that *N. galligena* destroys cellulose walls of invaded cortical tissue more rapidly than *G. perennans*. This may account for the extensive destruction of bark tissue that causes the characteristic concavity in the middle of *N. galligena* cankers.

Later in the development of *N. galligena* cankers the intercellular mycelium associated with the xylem fibres and sclerenchymatous bark fibres becomes strongly developed, growing spirally around small groups of fibres and giving a laced appearance to the walls of these elements. Although hyphae of *G. perennans* are found growing strongly between the bark fibres and the surrounding parenchymatous tissue, the mycelium does not appear to produce the characteristic laced effect on the fibre walls and no intercellular mycelium develops in the xylem.

Host reaction.

In both types of shoot infection the defence mechanism consists of (a) the formation of phellogens producing suberised barriers in the bark, and (b) the deposition of amorphous gum in the vessels of the xylem. The phellogens are, however, insufficient to provide permanent barriers against the spread of *N. galligena* and are successively penetrated by the mycelium in branches of all ages. Thus, in longitudinal sections through branch infections of *N. galligena*, successive phellogens mark the sites where the fungus has been temporarily checked. Although some callus tissue may be laid down behind the phellogens, in general the limits of *N. galligena* cankers are indistinct and the continued spread of the pathogen is indicated by small concentric ridges of wound wood. The repeated passing of wound phellogens by *N. galligena* again reflects the greater penetrative power of the enzymes produced by this organism, since, where similar branches are infected by *G. perennans*, the host generally has the ability to prevent further spread and a conspicuous callus ridge is laid down behind the first-formed phellogen. There is, however, little opportunity for the development of a similar, permanent callus around lesions of *N. galligena* (Plate XIII, Fig. 2).

Although renewed extension of lateral cankers of *G. perennans*, where healing normally takes place before the branch is completely girdled, may only be induced by artificially damaging the callus (5), recent work (4) has shown that natural penetration of wound barriers can take place in dieback infections of *G. perennans*. However, the mode of extension described above for *N. galligena* has so far been recorded only in summer pruning-cut infections of *G. perennans* on branches over 2 years of age (6).

PLATE XIII.



Fig. 2. Comparison of shoot infections caused by *N. galligena* (right) and *G. perennans* (left).
($\times 1\frac{2}{3}$).



Fig. 1. Comparison of shoot infections caused by *Nectria galligena* (left) and *Gloeosporium perennans* (centre and right).
(Nat. size).

PLATE XIV.



Fig. 1. Jamaican Lacatan bananas infected with *T. fructigena* after spraying a complete bunch with a spore suspension. Note random distribution of infections (x 1/3).



Fig. 2. Lacatan bananas after scratch-inoculation with spores of *T. fructigena*. Symptoms 5 days after inoculation, showing incrustation of conidiophores and conidia (x 1/3).

Reinfection of the healthy tissues lying behind suberised barriers is also effected by *N. galligena* by growth through the sclerenchymatous fibres of the inner cortex. Renewed advance of mycelium by way of the bark fibres appears to be of general occurrence in *N. galligena* cankers; it also seems to be the most frequent method by which reinfection takes place through the callus tissue below dieback infections of *G. perennans* on shoots of all ages (4). Crowdy (3) showed that hyphae of *N. galligena* also spread beyond the zone of blocked xylem vessels through the lumen of the xylem fibres: there is no evidence of fibre spread in the xylem of *G. perennans* cankers.

The above accounts, based on observations made during the course of work at Long Ashton, may prove a less reliable guide in distinguishing between cankers where the trees are growing on soils and under climatic conditions differing from those of North Somerset, or where more virulent strains of *G. perennans* are prevalent.

Summary.

The morphology of shoot infections of *Nectria galligena* is compared with that of *Gloeosporium perennans*. Differences in mycelial distribution and host response in the two types of lesion are discussed.

Acknowledgments.

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SOME OBSERVATIONS ON *TRACHYSPHAERA FRUCTIGENA* (TABOR ET BUNTING) WITH PARTICULAR REFERENCE TO JAMAICAN BANANAS.

By D. S. MEREDITH.*

Introduction.

Trachysphaera fructigena was first described in 1923 by Tabor and Bunting (1) as the organism causing mealy-pod disease of cocoa and coffee in the Gold Coast. During recent years the fungus has been recorded on bananas growing in West Africa (2, 3, 4). However, there appears to be no record of the fungus on these hosts in the West Indies.

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In the field, *T. fructigena* is reported (3) to produce symptoms similar to those of 'Cigar-end' disease caused by *Verticillium (Stachylidium) theobromae* (5). Tissues at the blossom-end of the finger darken and then shrink so that there is a folding of tissues. Diseased tissues become covered with conidiphores and the distal end resembles a burnt cigar tip. Brun & Merny (2), working on Cameroon Gros Michel fruit that had been shipped to Dieppe, observed a new form of fruit rot caused by *T. fructigena*. Infection was not always situated at the blossom-end and frequently spread completely throughout the finger.

The present paper describes observations on *T. fructigena* causing a rot of Jamaican bananas after arrival in the United Kingdom.

Occurrence on Jamaican and Other Fruit.

Although the fungus was often observed at English ports on newly arrived Gros Michel fruit from the British Cameroons and Fernando Po, it was never found on newly arrived Jamaican Gros Michel or Lacatan bananas. However, on three occasions, several bunches of infected Lacatan fruit were discovered inside ripening rooms. This fruit had ripened for 7-9 days and, in some instances, symptoms of disease were as advanced as those observed on mature Cameroon fruit that was already infected on arrival in the United Kingdom.

Rotting of Jamaican fruit was rarely of the typical 'Cigar-end' type, and infection occurred more or less at random along the finger. The disease was most frequent on the outer fingers of each hand and less frequent on the less exposed ones; also, in most cases, diseased tissues were centred about some form of old skin injury. Early stages of infection in green fruit were characterised by a buff-yellow or khaki discolouration of the skin, which became slightly depressed. As maturity was approached, rotting spread in all directions, the skin turning dark brown or black and becoming thrown into folds. Large numbers of conidia developed on the fruit surface to form a white or pinkish-brown mealy incrustation. In the final stage, the complete finger was considerably shrunk and presented a mummified appearance. Rotting of the pulp may be described as a dry rot, with a tendency to become tough and fibrous; considerable darkening takes place. In advanced stages of decay, several secondary organisms were associated with *T. fructigena*.

The symptoms described above are similar to those observed by Brun & Merny (2) on Gros Michel bananas.

Since it was known that the rooms that contained infected Jamaican fruit formerly contained severely infected Cameroon fruit, it seemed reasonable to suspect this as a possible source of inoculum. Of course, conveyor belts, railway trucks or lorries, on which fruit from both regions is transported, were also considered suspect. Further observations and experiments were initiated, therefore, in order to investigate the infection biology of *T. fructigena* on Jamaican fruit after discharge.

Experimental Infection.

Bunches of green Lacatan fruit were sprayed with a spore suspension of *T. fructigena*, obtained from infected fruit, until run-off occurred. The fruit was transferred immediately to ripening rooms and examined after 5 days ripening at 68°-70°F. On the 6 inoculated bunches, a total of 22 fingers were

infected. Infection occurred at the stem-end, in a median position or at the blossom-end and, in some cases, symptoms were as advanced as those observed on naturally infected fruit (Pl. XIV, Fig. 1); also, diseased areas were invariably centred about old skin wounds. Uninoculated controls, contained within the same rooms, did not develop disease symptoms. The experiment demonstrated the rapidity with which infection progresses, and also suggested that the fungus was acting predominantly as a wound parasite.

In another experiment, immature green Lacatan bananas were inoculated in various ways:

- (a) Fingers with persistent stigmas were selected and each stigma was inoculated by making a small incision and applying conidia;
- (b) Persistent stigmas were broken off and conidia applied to the fresh stigma scar;
- (c) The surface of the skin at the blossom-end of the fruit was lightly scratched and inoculated;
- (d) An inoculation similar to (c) was performed at a position about half-way along the finger;
- (e) As in (c) and (d) but on the finger-stalk;
- (f) Existing lesions, infected with *Gloeosporium musarum* (Cke. & Massee), were inoculated by painting-on conidia of *T. fructigena*;
- (g) Apparently healthy, undamaged skin was inoculated by applying conidia with a brush.

The fruit was ripened at 61°–70°F. for 5–6 days, after which time it was fully ripe. The results of inoculations are given in Table I.

TABLE I.
NUMBER OF INFECTED FINGERS (OUT OF 25) AFTER INOCULATION IN VARIOUS WAYS
WITH *T. FRUCTIGENA*.

Inoculation Type	No. of Successful Inoculations	Progress of Infection
(a)	20	Fair
(b)	25	Fair
(c)	3	V. Slight
(d)	15	Extensive (Pl. XIV, fig. 2)
(e)	11	V. Slight
(f)	0	—
(g)	0	—

An outstanding feature of these results was the rapid progress of infection after inoculation as in (d): stigma and stigma-scar inoculations showed a higher percentage of infection, but did not develop so rapidly. It is likely that skin tissues at the extreme blossom-end and in the finger-stalk offer greater resistance to infection than those at intermediate regions. Failure to infect unwounded tissues once more suggests that the fungus is a relatively weak parasite of harvested fruit.

An experiment was designed to test the ability of the fungus to infect unwounded fruit maintained under laboratory conditions. Immature fingers were detached from the main-stalk and surface-sterilized by immersion for 2 minutes in 0.1% mercuric chloride in 70% alcohol. A Van Tieghem cell was affixed about half-way along each finger and, after introducing 1 ml. of a dense

spore suspension of *T. fructigena*, it was sealed with a coverslip. The fruit was allowed to ripen for 14 days at 75°F., after which time there was no evidence of infection having occurred, even though a high percentage of spores had germinated. This finding lends further support to the suggestion that the fungus has only limited parasitic activity.

In another experiment, one finger was detached from each of 20 apparently healthy hands of immature Lacatan fruit. Each finger-stalk stump was inoculated immediately by applying conidia of *T. fructigena* to the freshly exposed tissues. After 5-7 days ripening at 62°-68°F., the fruit was fully ripe. In no instance did the fungus invade the cushion. This result is compatible with the observation that the fungus does not normally pass from one finger to another *via* the cushion or main-stalk.

Discussion.

Experimental inoculations carried out in ripening rooms have shown that *T. fructigena* readily infects immature Lacatan bananas when the skin tissue has been injured in some way. Symptoms of disease, which developed within a few days of inoculations, were often as advanced as those observed occasionally on Jamaican Lacatan fruit that had ripened in the United Kingdom. Since the fungus is not known to occur in the West Indies, and considering the fact that banana ships that carry Jamaican fruit do not normally visit West Africa, it seems reasonable to suggest that the natural infections were initiated after discharge from the ships. Ripening rooms in which diseased Jamaican fruit was found were formerly occupied by severely infected Cameroon or Fernando Po fruit, and these would appear to be one of the most likely sources of inoculum.

Although the quantity of diseased Jamaican fruit observed so far is commercially insignificant, it is obvious that the risk of such infection should be reduced. It is normal practice to wash out ripening rooms before introducing a fresh consignment of fruit; however, it is suggested that rooms formerly containing severely infected fruit should be disinfected more thoroughly. Similar considerations apply to chains and ropes by which stems are suspended. Also, all infected fruit should be removed as soon as it becomes evident, in order to minimize the amount of inoculum that may accumulate inside the room. Experimental inoculations indicated that the removal of infected hands, or even individual fingers, should not incur any risk of infection spreading to neighbouring fruit on the same stem *via* cushions.

The results of experimental inoculations suggested that the fungus is most active as a wound parasite. A similar conclusion was drawn by Brun & Merny (2), working on Gros Michel bananas from the Cameroons. It was noted in the present investigation that infections on Jamaican Lacatan fruit were usually associated with some form of mechanical injury to the skin, and in these instances the fungus was probably acting as a wound parasite. The failure to infect apparently healthy, unwounded tissues contrasts with the finding of Brun & Merny (2): they observed infection of unwounded Gros Michel bananas 9-10 days after inoculation. This may be partly due to differing varietal susceptibilities.

As pointed out by Brun (3), further investigation into the life history of *T. fructigena* is necessary in order to formulate suitable control measures.

Summary.

Trachysphaera fructigena (Tabor et Bunting) is recorded for the first time on Jamaican bananas of the Lacatan variety. The fungus was observed occasionally on almost mature fruit, which had been stored for several days inside ripening rooms in England, but not on newly arrived, green fruit. Symptoms of disease are comparable with those often observed on fruit from West Africa. Experimental inoculations showed that the fungus readily infects wounded, immature fruit, often resulting in the appearance of advanced symptoms after 5-7 days storage at 65°-75°F. All attempts to infect healthy unwounded fruit were unsuccessful. It is suggested that infection of Jamaican fruit was initiated by spores of *T. fructigena* that remained inside the ripening rooms after removal of severely infected West African fruit: infection may have occurred through existing skin injuries. Ripening-room hygiene is discussed briefly in relation to disease epidemiology.

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PRELIMINARY EXPERIMENTS ON FUNGITOXICITY AND PHYTOTOXICITY OF SOME PETROLEUM OILS.

By C. V. GOVINDASWAMY.*

Although mineral oils have long been used as herbicides, insecticides and spray supplements, it is only in recent years that they have been employed on a large scale as direct fungicides. Guyot & Cuillé (1) and Merny (2), in spraying trials on banana against the leafspot fungus *Mycosphaerella musicola*, found that a highly-refined mineral oil ('white oil') protected young leaves against infection and retarded the development of lesions on leaves already

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infected. Spraying with mineral oils is now extensively practised for the control of banana leafspot ; a wide range of oils has been employed, but there are frequent records of phytotoxic effects.

The experiments described in the present paper were made to obtain further information on the fungitoxicity and phytotoxicity of selected mineral oils. Using a number of pathogenic fungi commonly occurring in Britain, three aspects of the fungistatic action of the oils were explored : reduction in spore germination, checking of mycelial growth and interference with spore dissemination. The phytotoxic effects of the oils were assessed in glasshouse trials on a wide variety of crop plants, both temperate and tropical.

Materials.

Oils and emulsifiers. A white oil (P.31), spindle oil (JD.2) and diesel oil were chosen as representatives of the mineral oils employed in banana leafspot control. Emulsions of white oil and of spindle oil were prepared using 3% of the cetyl polyethylene glycol emulsifier CPGE.4. Diesel oil was emulsified with 3% of the closely-allied CPGE.6.

Fungi and media. Spores and mycelial cultures of *Alternaria tenuis*, *Botrytis cinerea*, *Cladosporium fulvum* and *Penicillium* sp. were derived from stock cultures maintained at Long Ashton. For experiments on spore dissemination, *Pseudopeziza ribis*, *Nectria galligena* and *Sclerotinia fructigena* were used *in situ* on their respective hosts : black currant leaves, apple shoots and apple fruits.

Mycelial growth studies were made using fungus colonies growing on an agar medium of the following composition : glucose, 40 g. ; peptone, 10 g. ; KH_2PO_4 , 6.8 g. ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5 g. ; CaCl_2 , 0.1 g. ; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.02 g. ; agar, 30 g. ; water, 1 litre.

Plants. The following plants, grown in pots, were used for phytotoxicity trials : cucumber (Butcher's Disease Resister), tomato (Radio), broad bean (Giant Windsor), barley (Plumage Archer), apple (Cox's Orange Pippin), black currant (Baldwin), gooseberry (Leveller), banana (Lacatan) and tea.

Methods.

Spore germination tests were made by the standard method described by the American Phytopathological Society (3). The slides carrying the fungus spores in selected concentrations of the oil emulsions (or emulsifiers) were incubated at 25°C. for 20 hr. and the percentage germination and germ-tube length then recorded. Determinations of the fungistatic effects of oil emulsions and emulsifiers on mycelial growth were made using the method of Byrde and Woodcock (4) in which successive measurements are made of the growth of the fungus on nutrient agar plates incorporating a range of concentrations of the material under test.

The effect of oil emulsions on the dissemination of conidia of the brown rot fungus, *Sclerotinia fructigena*, was estimated by dipping infected apples, bearing sporing pustules of the fungus, in the test liquids for 15 sec., then allowing to dry for 5 hr. The treated fruits were placed singly in glass funnels inserted in the necks of 250-ml. flasks and exposed outdoors to rain. The rainwater thus collected in the flasks, together with the rinsings obtained by washing out each flask with 5 ml. of a wetting agent (0.025% Ethylan), was

centrifuged 5 min. at 2,000 r.p.m. After decanting the supernatant fluid the sediment was taken up in 5 ml. distilled water and the spore concentration determined with a haemocytometer.

For testing the effects of oils on ascospore discharge from fructifications of the apple canker fungus *Nectria galligena*, small pieces of apple bark bearing mature perithecia were dipped for 1, 5 or 10 minutes in the neat oils or 25% oil emulsions. The treated material was then placed in moist chambers with the ostioles of the perithecia facing upwards; vaselined glass slides were supported approximately 0.5 cm. above the perithecia to catch the expelled ascospores. For the black currant leafspot fungus, *Pseudopeziza ribis*, the apothecia were similarly treated but arranged to discharge downwards on to a film of agar in a Petri dish.

In the phytotoxicity trials, whole plants or selected leaves were sprayed to run-off, using a hand atomiser, and kept in the glasshouse at temperatures that varied from 44° to 88°F. Observations were made on the 4th day from spraying and subsequently at 5-day intervals for 3 weeks. The intensity of foliage injury was recorded as nil, light, medium or severe, and these grades were assigned the values of 0, 1, 2, and 5 respectively.

Results.

EXPERIMENTS ON FUNGITOXICITY.

Effect on spore germination.

Table I shows the mean percentage germination of spores of the three fungi, *Alternaria tenuis*, *Botrytis cinerea* and *Cladosporium fulvum* in the presence of different concentrations of oil emulsions and emulsifiers.

TABLE I.
EFFECTS OF OIL EMULSIONS AND EMULSIFIERS ON THE GERMINATION OF
SPORES OF THREE FUNGI.

Material	Concentration	Percentage Germination		
		<i>A. tenuis</i>	<i>B. cinerea</i>	<i>C. fulvum</i>
P.31	1%	98	68	70
	5%	96	66	74
	10%	94	60	68
JD.2	1%	86	40	56
	5%	84	36	52
	10%	84	34	51
Diesel oil	1%	98	24	74
	5%	90	28	58
	10%	88	22	50
CPGE.4 CPGE.6	3%	96	80	94
	3%	95	81	88
Control		98	95	91

The results indicate that the spores of *A. tenuis* were little affected by any of the treatments. Germination of *B. cinerea* spores was reduced to one-quarter by diesel oil, and to one-third by JD.2 : P.31 and the emulsifiers slightly checked germination. Against *C. fulvum* JD.2 was a little more toxic than diesel oil : P.31 exerted only a slight check and the emulsifiers were non-toxic. Throughout the trial there was little difference between the results at the different concentrations, although the lowest germination figures were recorded at the highest concentrations of the emulsified oils.

Effect on germ-tube length.

Table II gives the mean germ-tube lengths measured for twenty-five spores in each of the incubation drops used for the germination counts. The results are expressed as percentages of the lengths recorded in the controls.

TABLE II.
EFFECTS OF OIL EMULSIONS AND EMULSIFIERS ON GERM-TUBE LENGTHS
OF THREE FUNGI.

Material	Concentration	Germ-tube length (as percentage of control)		
		<i>A. tenuis</i>	<i>B. cinerea</i>	<i>C. fulvum</i>
P.31	1%	102.1	17.7	26.7
	5%	100.4	16.2	33.0
	10%	101.3	13.1	28.1
JD.2	1%	93.4	22.9	17.3
	5%	85.9	18.2	16.3
	10%	88.5	15.3	13.9
Diesel oil	1%	74.2	4.3	19.2
	5%	72.7	2.9	14.1
	10%	66.2	2.9	11.7
CPGE.4	3%	101.6	31.0	42.7
CPGE.6	3%	102.0	23.3	30.1
Control		100.0	100.0	100.0

In *A. tenuis*, germ-tube length was affected only by diesel oil. For *B. cinerea*, germ-tube growth was severely checked by all the oils and emulsifiers, diesel oil having the most effect. A similar result was shown by *C. fulvum* but the inhibitory effect, except in the case of JD.2, was slightly less.

As in Table I, no marked difference was obtained by varying the concentration of any one oil.

Effect on mycelial growth.

Mycelial growths of *A. tenuis*, *B. cinerea* and *Penicillium* sp. were recorded as the increase in mean colony diameter from the 2nd to the 4th day. In the slower-growing *C. fulvum* the records were taken between the 3rd and the 5th day. Four replicate plates were used for each treatment and these were randomized in the incubator as suggested by Clarke (5). The results are assembled in Table III.

TABLE III.

EFFECTS OF OIL EMULSIONS AND EMULSIFIERS ON MEAN INCREASE IN DIAMETERS
OF COLONIES ON NUTRIENT AGAR.

Material	Concentration	Mean 48-hr. increase in colony diameter (mm.)			
		<i>A. tenuis</i>	<i>B. cinerea</i>	<i>Penicillium</i> sp.	<i>C. fulvum</i>
P.31	1%	17.0	34.4	13.4	8.0
	5%	16.8	25.3	11.8	7.3
	10%	12.8	20.9	9.9	7.4
JD.2	1%	17.0	50.9	15.4	6.8
	5%	16.5	32.3	12.5	7.3
	10%	14.6	25.4	11.9	—
Diesel oil	1%	17.3	12.8	10.5	5.6
	5%	14.0	10.0	10.6	4.9
	10%	13.3	9.3	10.3	5.8
CPGE.4	3%	19.1	40.1	17.3	6.5
CPGE.6	3%	19.8	17.5	11.8	7.1
Control		27.4	63.0	17.4	9.9
Sig. diff. at $P=0.1$		4.5	5.2	2.1	1.0

All the oils, including P.31, were moderately effective in checking the mycelial growth of *A. tenuis*. Against *B. cinerea* the results were similar to those given in Table II; diesel oil showing a strong inhibitory effect and CPGE.6 also causing a marked check. The results with *Penicillium* resembled those for *A. tenuis*. Against the slow-growing *C. fulvum*, diesel oil was the most effective. It was noted that no inhibition of the sporulation of *C. fulvum* was caused by any of the oils.

Effect on dissemination of conidia.

The results on the dissemination by rain of conidia of the brown rot fungus, *Sclerotinia fructigena*, from pustules on oil-dipped apples are given in Table IV.

TABLE IV.

EFFECTS OF OIL EMULSIONS AND EMULSIFIERS ON THE DISSEMINATION
OF *S. FRUCTIGENA* CONIDIA BY RAIN WATER.

Material used as dip	Concentration	No. of spores per ml. removed by rain	% of control
P.31	5%	20,000	3.2
Diesel oil	5%	20,000	3.2
CPGE.4	3%	190,000	30.2
CPGE.6	3%	142,000	22.5
Water		205,000	32.4
Control		630,000	100.0

The great reduction in the number of spores disseminated, following the treatment with oil emulsions, will be seen. The much smaller effect of the emulsifiers was approximately equal to that of a water dip and probably reflected the mechanical removal of all the existing mature spores before the infected fruit was subjected to rain.

Effect on discharge of ascospores.

When fruit-bodies of *Nectria galligena* on apple shoots, or of *Pseudopeziza ribis* on black currant leaves, were dipped in oil emulsions at 25%, or in neat oils, there was no reducing effect on the discharge of ascospores, provided that excess oil on the surface was removed. If, however, a film of oil was allowed to remain on the surface of an infected black currant leaf it delayed the ascospore discharge of *P. ribis*.

EXPERIMENTS ON PHYTOTOXICITY.

Table V summarizes the records of injury to the various crop plants subjected to the oil emulsion sprays. Preliminary experiments showed that the emulsifiers used alone were not phytotoxic.

TABLE V.
PHYTOTOXIC EFFECTS OF OIL EMULSIONS ON VARIOUS CROPS.
(Records of glasshouse tests).

		Crop								
		0=Healthy	1=Light injury	2=Medium injury	5=Severe injury					
Material	Concn.	Cucum- ber	Toma- to	Bean	Barley	Goose- berry	Black currant	Apple	Tea	Banana
P.31	1%	0	0	0	0	0	0	0	0	0
	5%	1	1	0	0	1	0	1	0	0
	10%	2	2	5	0	5	1	5	1	0
JD.2	1%	1	2	1	0	1	0	1	0	0
	5%	2	5	2	0	5	1	2	1	0
	10%	5	5	5	1	5	5	5	5	2
Diesel oil	1%	0	0	0	0	0	0	0	0	0
	5%	1	1	1	0	2	1	1	1	0
	10%	2	2	5	1	5	2	5	2	2

The results, taken as a whole, confirm expectations that the highly-refined white oil (P.31) would prove much less damaging than the cheaper spindle oil; the effect of the diesel oil being intermediate. Of the crops tested, barley and banana were particularly resistant to oil injury; gooseberry and apple were the most susceptible. The high rating of gooseberry is probably influenced by the fact that the variety employed, Leveller, is one that is specially sensitive to spray injury. In these phytotoxicity tests—in contrast to those on fungitoxicity—the effect of concentration of the oil was strongly marked. The 10% emulsions of all the oils were damaging to all the crops, with the exception of 10% P.31 applied to banana or barley. At 1%, neither P.31 nor diesel oil caused any injury and at 5% the injury caused by these two oils was slight.

Discussion.

In the evaluation of fungicides, much importance is rightly attached to spore germination assays. In some circumstances, however, substances which do not strongly inhibit spore germination may be of value as fungitoxic materials by their action in depressing sporulation, preventing spore dissemination or checking mycelial growth. Such effects might influence the spread and development of plant-pathogenic fungi.

The results obtained in the present investigation suggest that the mineral oils fall into the category of materials that are not strongly fungicidal but are fungistatic to a greater degree than has hitherto been assumed. Within the limited field covered by these experiments it is clear that sensitivity to oils varies widely between fungus species and between different stages of the same fungus. In spore germination tests and comparisons of germ-tube length, *Alternaria tenuis* was shown to be much more resistant than *Botrytis cinerea* or *Cladosporium fulvum* to the effects of oil emulsions. In comparisons of mycelial growth, however, all these fungi showed the retarding effect of oil emulsions at all concentrations down to 1%.

The effect of oil in preventing the release of conidia by rain falling on spore pustules of *Sclerotinia fructigena* indicates a fungistatic mechanism that has not previously been recorded. This action may be of practical significance in checking the spread of infections that are dependent on rain-splash dispersal of conidia.

The main point of interest in the phytotoxicity trials is in the comparisons made on temperate and tropical crop plants growing in the same environment. The wide range in sensitivity between these plants was apparent and it was noteworthy that banana leaves were much more resistant to oil damage than those of temperate fruit plants. Further trials, in which the undiluted oils will be applied to give a standardized deposit pattern, will be reported later.

Summary.

- (1) In experiments with emulsions of three grades of mineral oil it was shown that 1% diesel oil and spindle oil markedly reduced the germination of spores of *Botrytis cinerea* and caused some reduction in spore germination of *Cladosporium fulvum*. White oil, up to 10%, had little inhibitory effect on spore germination of these species. Germination of spores of *Alternaria tenuis* remained practically unaffected by any of the oil emulsions tested.
- (2) The effects of the emulsions on the growth of germ tubes of the above three fungi followed the same trends as in the germination tests.
- (3) All the emulsions had an inhibitory effect on the mycelial growth of the three fungi and of *Penicillium* sp.; they did not check sporulation of cultures of *Cladosporium fulvum*.
- (4) Conidial pustules of *Sclerotinia fructigena* on oil-treated apple fruits showed considerable resistance to washing-off of the spores by rain.
- (5) The emulsions were found to have no adverse effect on the discharge of ascospores of *Nectria galligena* and *Pseudopeziza ribis*.

- (6) The phytotoxic effects of the emulsions were tested in the glasshouse on nine crop plants. At 1% concentration, the white oil and diesel oil were harmless to all crops tested; at 5%, the spindle oil was generally more phytocidal than the other two; at 10%, some damage was caused to every crop by all the oils, except by white oil on barley and banana. These two crops were markedly resistant to damage; gooseberry and apple were the most susceptible.

Acknowledgments.

The author is greatly indebted to Dr. C. G. L. Furmidge, Dr. A. T. K. Corke and Mr. C. S. Gundry for the provision of materials and to Mr. G. M. Clarke for help with the statistical analyses. Mr. R. W. Marsh, who suggested the problem, and Dr. R. J. W. Byrde gave valuable guidance and assistance throughout this work.

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A PRELIMINARY NOTE ON THE LEAF HOPPERS OCCURRING ON APPLES AT LONG ASHTON.

By S. H. BENNETT.

Although considerable fluctuations in the population of hoppers occur during a season and from year to year, the damage caused by them in some seasons is considerable. The female hoppers usually lay their eggs in the young shoots in late summer and autumn and these eggs overwinter, hatching in late spring or early summer. There are five nymphal instars, and in some species the resultant adults may produce a second generation. The eggs of the second generation are usually deposited in the tissue of the under-surface of the leaves, and resultant adults appear in late summer.

All stages of the insect feed from the under-surface of the leaves on the mesophyll tissue, and this results in a silvering of the foliage. The loss of chlorophyll must in itself be detrimental to the tree, and in seasons of severe attack damage may be sufficiently important to warrant control measures, though these are rarely carried out as the symptoms are recognized too late. As the leaf hoppers are suspected vectors of viruses of top fruit their role may be of considerable importance; therefore more information on their biology and distribution is required.

The leaf hoppers are a difficult group of insects to study, as species can be correctly named only from the morphological characters of the aedeagus. This makes field identification uncertain and the work of sorting out mixed populations laborious.

Experimental.

During 1958 detailed observations of hopper populations were made on a 7½ acre bush cider plot planted at Long Ashton in 1935-36. The plot layout was two 8-row blocks, with alternate rows of the varieties Sweet Alford and Stoke Red on Crab rootstock and a double barrier row of Laxton's Superb on M II. The planting distance for the cider varieties was 40' between the trees and 20' between the rows, with a 60' gap between the next two rows; the Laxton's Superb plant was 20' between the trees and 40' between the rows. The orchard was sprayed on May 13th with BHC/DDT emulsion + ¾% lime sulphur + nonyl sodium sulphosuccinate; no further applications of insecticide or fungicide were made.

Method of Sampling.

Counts were made at frequent intervals during the season; the method of sampling was to record the total number of both hopper adults and nymphs on a 40-leaf sample, taken in eight groups of five leaves each, from each tree. All samples were taken at shoulder height from eight quadrants round the tree, four being on the outside branches and four nearer the main trunk. Adults and nymphs were noted separately at the time of recording in the field, but division into instars and species was not made at this stage. After recording each tree by this method, a large random collection of nymphs and adults was made; these were separated in the laboratory into species and instars, and this information was used to assess the nymphal stages and species in the field counts.

This method of sampling is more satisfactory for the less mobile nymphal stages than for adults. Adults take to the wing very readily when the leaves are disturbed, and the speed at which they do so varies. Under certain climatic conditions the adults may become very active, the degree of activity depending on species and on the time of day. By turning leaves over carefully to avoid shaking, and by taking only five leaves in a particular area, errors can be minimized; but if large numbers of adults were present the method would probably be inadequate. With the population density existing in 1958, however, the method seemed satisfactory.

Results.

Only four species of hoppers were recorded during the season. In order of prevalence these were *Erythroneura alneti* (Dahlb.), *Typhlocyba froggatti* Baker, *T. debilis* Dgl., and *T. rosae* L. The most interesting of these species is *E. alneti*, of which no previous record on apples has been found, its normal hosts being alder and elm. Young nymphs of this species were found early in the season; hence its complete life cycle occurred on apples and it would appear, therefore, to have changed its habitat.

The first nymphs were recorded on May 28th and pairing adults on July 24th. Oviposition in the bark was first noted on August 6th but it was not until the beginning of September that numbers of females were observed egg-laying.

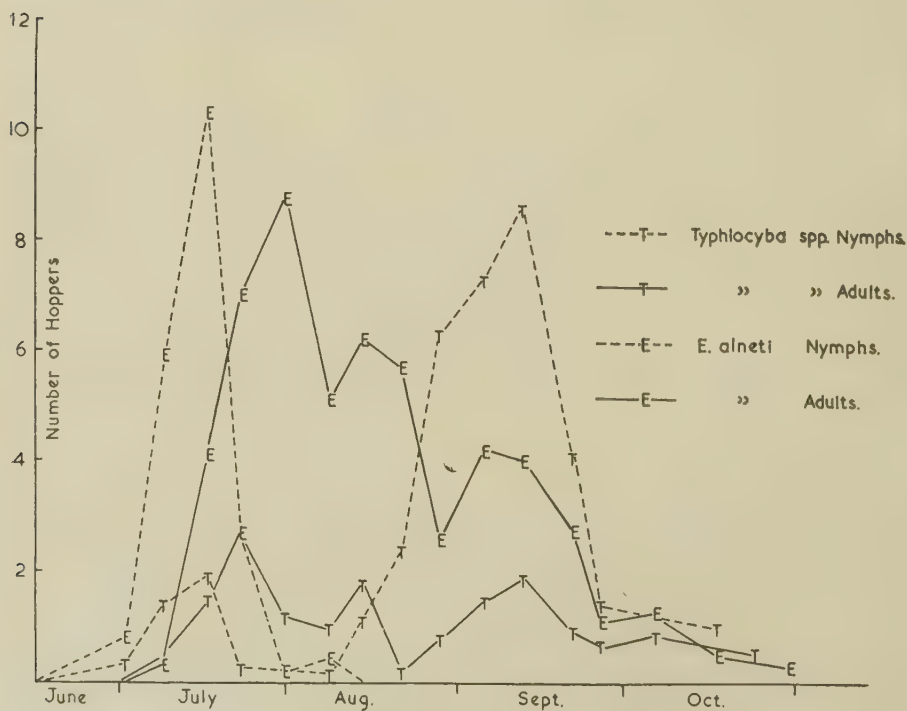


Fig. 1. Number of 5th instar nymphs and adults of *E. alneti* and of three species of *Typhlocyba* per 40-leaf sample on Laxton's Superb.

The population of 5th instar nymphs and adult hoppers per 40-leaf sample on the variety Laxton's Superb is shown in Fig. 1, in which the three *Typhlocybid* species have been grouped together. It will be seen that *E. alneti* is univoltine and that the Typhlocybrids have two generations. It should be noted that this is a composite graph of the three species: more data on the life histories of the individual species are required to determine whether all have two generations and, if so, the relative importance of each generation within the species.

Adults of *T. froggatti* and *T. debilis* were recorded from early July until late October: whilst the former was the predominant species in July and August, after August *T. debilis* predominated. A few isolated females of *T. rosae* were taken early in the season but it was not until late August that both sexes were recorded.

The total population of hopper nymphs was comparable on a time axis on all three varieties of apple (Fig. 2). The differences in numbers per 40-leaf sample from the three varieties is not necessarily due to a varietal preference by the leaf hoppers but may be due to variations in the area of the leaves of the different varieties.

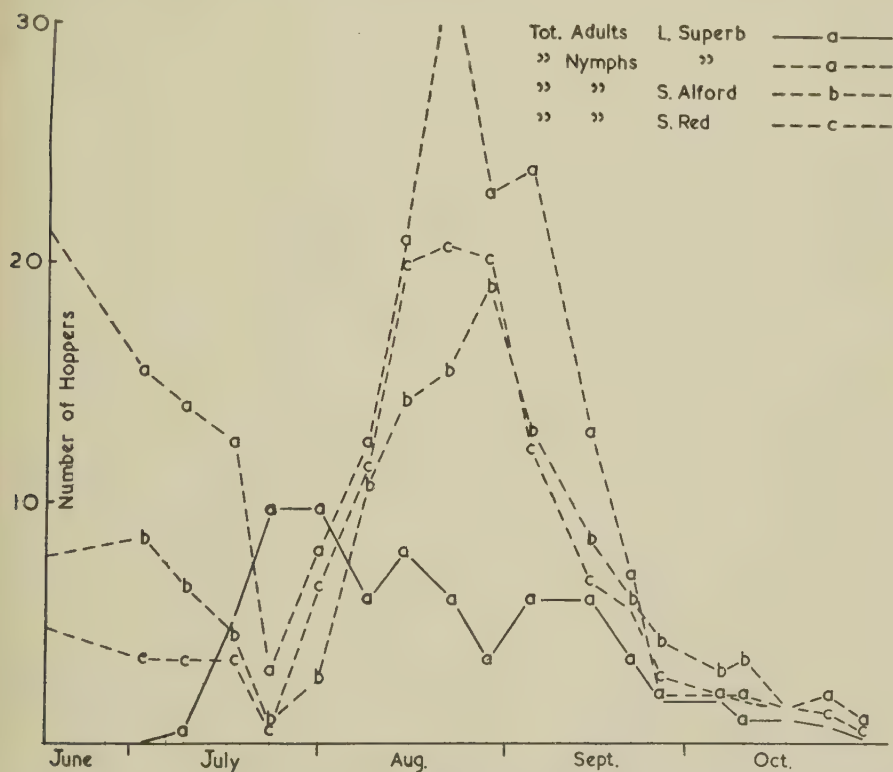


Fig. 2. Total number of hopper nymphs per 40-leaf sample on Laxton's Superb, Sweet Alford and Stoke Red and total hopper adults per 40-leaf sample on Laxton's Superb.

Acknowledgments.

Thanks are due to Miss P. Herrington for help in field and laboratory work and to Mr. G. M. Clarke for help in evolving a sampling method.

SPRAY APPLICATION PROBLEMS: L.

THE USE OF FLUORESCENT MATERIALS AS TRACERS IN SPRAY LIQUIDS:

EXPERIMENTS WITH ABACA (*MUSA TEXTILIS*, NEE).

By H. R. MAPOTHER and ANN R. TAPSCOTT.

The use of fluorescent tracers for determining the degree of spray cover was suggested in 1955 by Sharp (1); deposits from spray liquids containing such tracers can be examined visually in ultra-violet light, or photographed if a permanent record is desired.

After preliminary trial at Long Ashton in 1957 the technique was applied during an investigation of the control of the aphid vector of Bunchy Top disease of abaca in North Borneo in 1957/58.

Method.

Tracers. Of a number of materials examined for their suitability as tracers, two materials, Saturn Yellow and zinc 8-hydroxyquinolate, were found to give exceptional brilliance and a suitable colour contrast on foliage. Both are available with a uniform particle size of approximately 10 microns for the zinc salt and 4 microns for Saturn Yellow.

Formulation. Maximum intensity of fluorescence is given when the tracer is formulated as a suspension (Pl. XV, Figs. 1 and 2). Some aggregation of individual particles may be caused by certain wetters, although the degree of aggregation may not be great enough to preclude their use, e.g., the aggregation of Saturn Yellow by Manoxol N (sodium dinonyl sulphosuccinate).

Objection can be made to the use of a suspension since the tracer may not be in the same phase as a soluble insecticide or fungicide. There may then be differences in the behaviour of the two deposits; either may, for instance, be preferentially leached by rain or spray run-off, or have a different power of adhesion to foliage.

It has been found possible to emulsify Saturn Yellow from solution in methyl salicylate or dimethyl phthalate with a non-ionic emulsifier (cetyl alcohol/ethylene oxide condensate). Several tracers available as inks for flaw detection in metals, such as Britmor, can also be emulsified.

A suitable concentration of tracer for a suspension is about 0.1% and is prepared by blending the required weight with 2-3 ml. water and one drop of wetter. It is then added, with stirring, to the bulk of the liquid, which should be shaken at intervals during use.

With emulsions, which give a less intense brilliance, the concentration of tracer must be increased. Maximum intensity is obtained with Saturn Yellow at 0.4%, although a concentration of 0.2% is generally sufficient (Pl. XV, Figs. 3 and 4).

Photographic Records.

The photographs shown (Plates XV and XVI) were taken with the apparatus finally chosen, which consisted of a 2¼" sq. single-lens reflex camera, mounted on a travelling stand similar to an optical bench. Extension tubes were frequently employed. To prevent ultra-violet light affecting the negative, a

filter must be used; suitable ones were the Ilford 110 (Micro 4, Minus Blue) which is gelatine, and the Actina Dark Yellow $\times 4$ which is all-glass. The source of ultra-violet light was a Hanovia Model II lamp. The sample, if paper or foliage, was held against a small vertical easel, by means of glass if the entire sample was required, or by a sheet of aluminium with a circular aperture of suitable diameter if only a portion was needed.

To isolate an object from its background, the latter was treated with a 0.025% aqueous solution of Fluolite C which photographs white in ultra-violet light. Using Ilford HP3 film, exposures were in general about 1 minute at f 8.

Applications and Limitations.

To study the behaviour of tracers under natural conditions of weathering, shoots and clusters of fruitlets of Cox's Orange Pippin and Laxton Superb apples and Williams' pear were dipped, without removing from the tree, in 0.05% DDT emulsion containing 0.019% Manoxol N and 0.1% Saturn Yellow as a suspension. Treatment was made in June and samples were withdrawn at intervals to mid-August.

After more than two months' weathering, during which the deposits were subjected to 6.31 in. of rain (normal rainfall 6.6 in.), slight fluorescence was present and between 5% and 15% of the original DDT deposits remained. As might be expected from their structure, a heavier deposit occurred on the under surface of apple leaves than on the upper surface, and concentration of deposit took place along the veins (Pl. XV, Figs. 1-4).

Attempts to correlate fluorescence with the quantity of DDT present on the foliage were made by counts of tracer particles and by chemical estimation of DDT on discs cut from leaves subjected to dipping or spraying on one side only. Replicates of counts were found to vary widely and no quantitative relation could be established. This may be partly accounted for by the tracer's occurring both as dispersed particles and as aggregates which differ so greatly in intensity that limits are imposed on photographic registration.

Investigations of the rate of leaching of deposits on leaves by water sprays showed initial DDT deposits to be higher than normal, probably because of occlusion by tracer aggregates. These aggregates appear to be leached at the same rate as the DDT and practically the whole loss occurs during the first five minutes spraying. The following values for DDT ($\mu\text{g./sq. cm.}$) were obtained on apple leaves.

<i>Duration of water spray (min.)</i>	<i>DDT emulsion</i>	<i>DDT emulsion + tracer</i>
0	2.0	4.3
5	—	1.3
10	1.0	1.2
60	0.9	1.2

It is apparent that, in its present form, the tracer technique cannot be used quantitatively. It does, however, afford a valuable method for assessing the initial degree of cover given by a spray. It has the great advantage of giving a picture of the distribution of the material over the spray target which can be rapidly seen by merely holding in ultra-violet light. The deposit can be photographed if a permanent record is desired.

It can, to advantage, be combined with chemical estimation since the latter does not differentiate between equal deposits resulting from an overall cover of small droplets or irregular cover from a few large drops.

Experiments with Abaca.

Abaca or Manila Hemp (*Musa textilis* Née) was planted in some of the areas of rich volcanic soils of North Borneo by the Japanese before the second World War. During and after the war the estates were neglected and ravaged by disease. In 1951 full control was assumed by the Colonial Development Corporation; Borneo Abaca Ltd. was constituted and re-planting and disease-control programmes were commenced. By 1958 the estates contained 3,700 acres of hemp and exported 2,739 tons of finished fibre valued at 3,500,000 dollars. These estates of Borneo Abaca Ltd. are the only ones in the Commonwealth producing abaca.

Abaca is of the banana family and the valuable fibre runs the length of the pseudostem in the leaf sheaths. It is subject to the severe virus disease, Bunchy Top, which causes premature opening of leaves leading to a rosetted form of crown and stunted pseudostem. The disease also occurs in wild and edible bananas (*Musa* spp.), is transmitted by the aphid, *Pentalonia nigronervosa* Coq. (2, 3, 4, 5,) and, if unchecked, rapidly spreads to the point of devastation.

Abaca is planted on the square, $11\frac{1}{2}$ ft. $\times$ $11\frac{1}{2}$ ft., and because of regeneration by suckers each plant soon becomes surrounded by stems, the unit being termed a "mat" (Plate XV, Fig. 5).

Rigorous inspection is in operation on the estates and any mat carrying a plant exhibiting symptoms of the disease is immediately rogued and sprayed, together with adjacent mats to a depth of three. Prior to the present experiments 0.5% BHC Dispersible Powder (0.0325% gamma isomer) and a proprietary wetter were used, and spraying was repeated five times at weekly intervals. The combined cultural and spraying treatment appeared to be effective in controlling spread of the disease but was expensive.

Experiments were conducted to investigate the possibility of effecting practical and economical improvements in control technique by consideration of the following factors.

(a) Efficiency of wetter.

Abaca leaves are not easily wetted, particularly the young unfurling leaf which is a major aphid site. The wetter then in use at 0.08% was found to be ineffective unless its concentration were raised to 0.7%. Manoxol N at 0.05% was found to be eminently satisfactory and has been adopted.

(b) Efficiency and residual effect of insecticide.

Comparative trials of a number of insecticides were made on potted abaca plants artificially infested with aphids. It was found that 0.1% malathion and 0.05% endrin as emulsions gave 100% initial kill compared with an average of 80% for BHC Dispersible Powder (0.0325% gamma isomer). From the point of view of residual effect there was little to choose between the three treatments. Since, however, it appeared prudent to wipe out the initial population containing infective aphids at the time of roguing, and in view of its low mammalian toxicity, malathion is now in use.

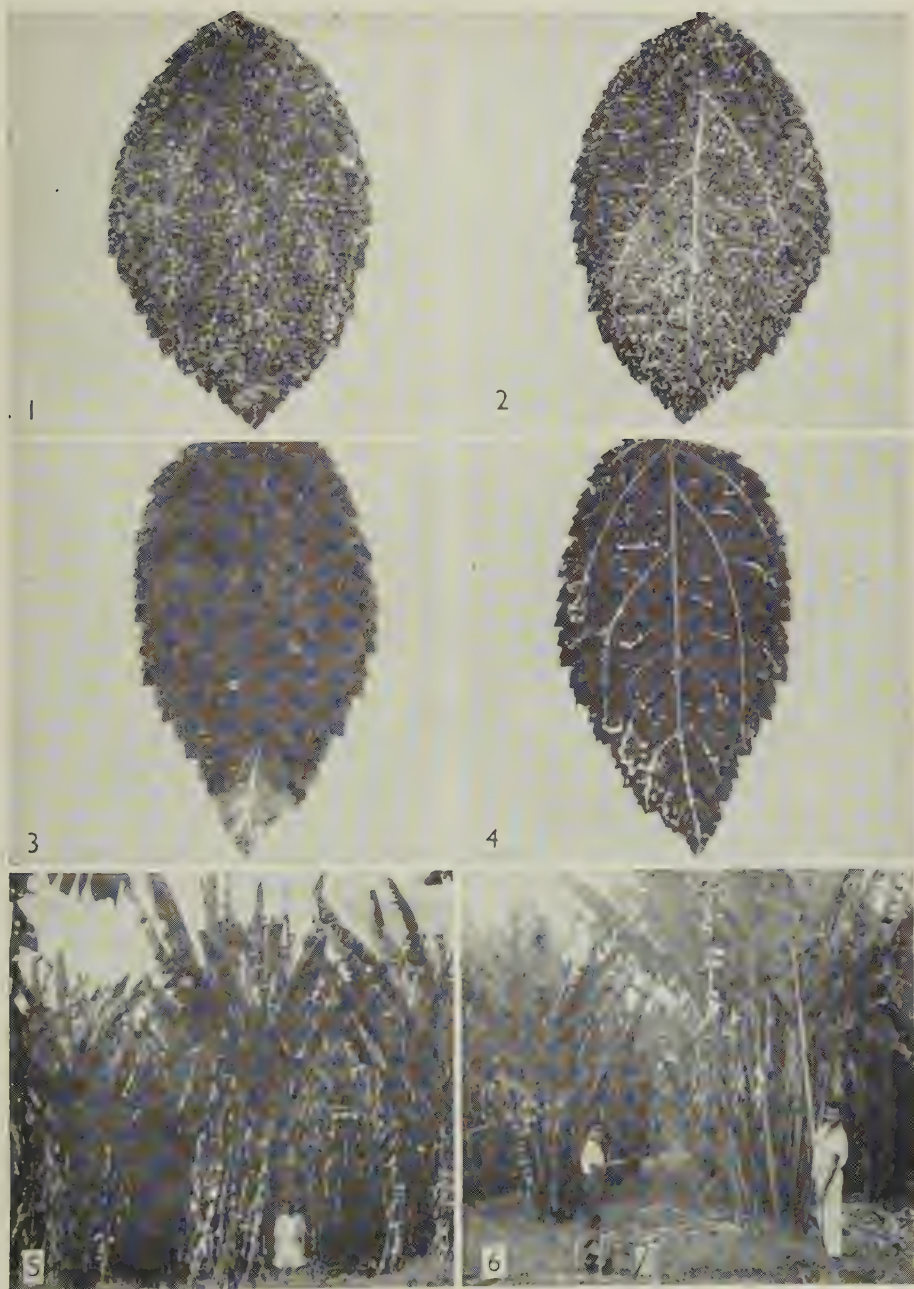


Fig. 1. Apple leaf dipped in 0.1% suspension of Saturn Yellow ; upper surface.
Fig. 2. As *Fig. 1* ; under surface.
Fig. 3. Apple leaf dipped in 0.2% emulsion of Saturn Yellow ; upper surface.
Fig. 4. As *Fig. 3* ; under surface.
Fig. 5. Mature abaca (Manila Hemp).
Fig. 6. Lightweight power-operated sprayer in use on abaca.

PLATE XVI.

Fluorescent deposits on abaca from various spraying machines.



Fig. 1. Large drops.
Fig. 3. Streaming.
Fig. 5. Insufficient cover.

Fig. 2. Splashing.
Fig. 4. Excessive cover.
Fig. 6. Adequate cover.

(c) *Frequency of spraying.*

Frequency of spraying is dependent on the duration of the residual effect of the insecticide, although complicated by the rapid growth rate of abaca whereby fresh tissue becomes available for infestation. From measurements of residual effect it was considered that four sprayings with malathion at 10-day intervals would be as effective as the normal practice of six sprayings with BHC at 7-day intervals. These two treatments are at present being compared on a field scale of several thousand acres.

(d) *Depth of adjacent mats sprayed.*

A limited study of the movement of aphids from infested mats to adjacent clean mats suggested that virtually no movement took place until about ten days had elapsed. It was therefore considered that the depth of spraying round a rogued mat could be reduced from three to two mats, which would represent a reduction in spray materials of 49%. This is to be the subject of large scale trial when the malathion v BHC trials have been completed.

(e) *Efficiency of spraying.*

Abaca has an upright habit of growth. The leaves on a mature plant may reach to over 20 ft., with the base of the growing point 12-15 ft. above ground level; on the same mats there will be suckers at various stages of growth. The main aphid sites are on and in the growing point, the first two leaf sheaths and the base of the stem, for all stages of growth from sucker to mature.

Two types of machine were in use on the estates: pneumatic knapsack sprayers and portable power machines of Australian origin; the latter were unduly heavy for their type. Two spray nozzles were employed to give "long" or "short" throw, the former being used to reach into the crowns.

It was decided to modify this technique by using a long lance (10 ft. aluminium) to lift an orthodox nozzle into the crowns of the highest plants and a short lance (3 ft. brass) to spray all other parts, each lance carrying 60 ft. of hose and both operating at 75 p.s.i. from a lightweight portable power machine (6). (Pl. XV, Fig. 6). Saturn Yellow was incorporated as a 0.1% suspension in the spray liquid and used to assess the cover given by each type of machine. The fluorescence on representative portions of leaves was recorded photographically.

It was found that the long-throw nozzles, tending towards jets, splashed rather than sprayed the foliage (Pl. XVI, Figs. 1 and 2). Any excess spray liquid on the upper surface of leaves will find its way by gravity into the leaf sheath, an important aphid site. The long-throw nozzle, because of projection from beneath the crown, frequently gave very little deposit on the upper surface whereas the unimportant under surfaces were heavily treated. A very great improvement was apparent from the use of long lances. The types of deposit obtained are shown in Pl. XVI, Figs. 3-6.

The use of the lightweight power machine with one long and one short lance has now been adopted on the estates.

Although chemical estimations were carried out, no deductions concerning the type of deposit could be made such as were immediately apparent from the tracer technique.

Summary.

The use of fluorescent materials in spray liquids affords a rapid and simple method of determining the type and degree of deposit obtained on foliage.

The method, in its present form, is not quantitative.

The technique has been used with success to investigate the type of spray cover given by different types of spray equipment on abaca in North Borneo.

Acknowledgment.

The authors are greatly indebted to L. N. Staniland, Esq., N.A.A.S., S.W. Region, Bristol, for much advice and for the supply of a number of fluorescent materials.

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STUDIES ON LIQUID SURFACES :

I. THE VIBRATING JET METHOD FOR THE DETERMINATION OF DYNAMIC SURFACE TENSIONS.

By W. D. E. THOMAS and L. POTTER.

Introduction.

Problems of emulsification, wetting and foaming are frequently met in agricultural spraying. Such processes are largely governed by reactions that occur at boundaries between two or more phases ; the study of boundary phenomena is therefore of considerable practical importance. In many instances much of the information necessary for the elucidation of these problems is obtainable from a knowledge of surface tensions. The term ' surface tension ' is numerically equivalent to, and is expressed in the same units as, the more acceptable thermodynamic property ' surface energy.' Within the bulk of a liquid the resultant force on every molecule is zero, since the attraction by the surrounding liquid is uniform in every direction ; consequently free molecular movement within the liquid is possible without change of energy. At the

surface, however, there is a strong inward-pulling force acting on each molecule, causing the surface to contract to its minimum area. To extend this surface therefore requires additional energy and this is termed the 'surface energy.'

Surface active compounds in aqueous solution have the property of reducing the surface energy: they generally contain a water-repelling hydrocarbon chain with an attached hydrophilic or soluble group and function by reducing the dissymmetry of the surface forces.

In a freshly formed surface of a dilute aqueous solution of a surface active compound, the surface tension at the instant of formation will be only slightly lower than that of pure water, since relatively few solute molecules will be present on the new surface. Immediately following the formation of the surface, however, migration of solute molecules will occur and will continue until adsorption is complete and the surface has a minimum energy corresponding to the equilibrium (or static) surface tension. The interval between the formation of the surface and the attainment of adsorption equilibrium is termed 'aging' and the value of the surface tension at any time during this interval is referred to as a 'dynamic tension.' Such values of the tension must always therefore be associated with the corresponding age of surface. Aging is complete within a few thousandths of a second in some instances but in others it may be prolonged for hours or even days, depending on the identity and concentration of the surface active compound used. Whilst the physico-chemical properties of interfaces in equilibrium have been extensively studied, very few workers have investigated the rates at which this equilibrium is reached.

The vibrating jet method provides the most reliable means of determining dynamic tensions for surfaces that attain equilibrium tension within the range 0.001–0.02 seconds after formation. Work on the possibilities of this method has been carried out before studying aqueous solutions of surface active compounds.

The Vibrating Jet Method.

Bidone in 1830 (1) and Magnus (2) were the first to observe that when a liquid issues at a constant rate from an elliptical orifice a series of stationary waves, vibrating alternately in a horizontal and a vertical plane, is formed in the stream. Buff (3) attributed this behaviour to surface tension effects and Rayleigh (4) and Pedersen (5) adapted the phenomenon to measurement of tensions. Bohr in 1908 (6) studied the hydrodynamics of jet streams and developed a comprehensive equation relating the surface tension along the stream to several parameters, the principal ones being: (i) the distance between two adjacent wave crests (wavelength); (ii) the stream radius—a function of the maximum and minimum cross section of the same wave; and (iii) the rate of flow of the stream. The surface age at any point along the stream may be calculated from (ii) and (iii), the age being zero at the orifice tip. A series of corresponding surface tension-surface age values may thus be obtained using the same stream. For a pure liquid such as water the method would be expected to give rise to one value of the tension—that obtained by static methods—and the accuracy with which results from the vibrating jet approach the accepted static tension value of water (72.8 dynes/cm. at 20°C.) is a valuable guide to the reliability of the method. Stocker (7) used the Bohr

equation and introduced an ingenious optical device for measuring the wavelength. Addison (8) avoided measuring the stream radius by using an empirical equation when determining the dynamic tensions of aliphatic alcohols. More recent work has been carried out by Rideal and Sutherland (9, 10) and by Defay and Hommelen (11, 12). There is disagreement between the results obtained by the last three groups of workers and in their interpretation of the mechanism by which surface adsorption occurs. In the present work an attempt was made to simulate Addison's method but, because of the assumptions necessary, it was abandoned in favour of a procedure based on that of Bohr and Stocker.

Experimental.

Preparation of orifices. After experimenting with several procedures for making orifice tubes, the following was found to give a fair proportion of satisfactory tubes. Two lengths of $\frac{3}{8}$ " diam. pyrex glass tube mounted in the chucks of a glass lathe were heated and joined. When the bore at the junction had become reduced by heat to capillary dimensions it was gently compressed to give an elliptical cross-section. After cooling, the tube was cleanly cut where the bore was narrowest, producing two orifice tubes. Each tube was about 7 cm. long and when in use was attached by a rubber sleeve to the outlet tube from the aspirator or pressure flask (see below).

Arrangement for constant flow. Two procedures were used for maintaining a constant rate of flow from the orifice :

(a) For pressures up to 10 cm. mercury the liquid was allowed to flow from an aspirator into which air was admitted through a capillary tube (diam. 0.08 cm.). Provided the capillary tip was below the liquid level in the aspirator, the flow rate was independent of the level.

(b) For higher pressures (up to 60 cm. mercury) the orifice was attached to a specially designed pyrex flask containing the liquid. Pressure was maintained constant (as read on a sensitive manometer) by manual control of the air stream from a reservoir, using a needle valve.

Measurement of outflow rate. This was obtained by allowing the stream to flow into a weighed container for a known time interval. Movement of a control shutter device operated a mains frequency counter, permitting time intervals (of about a minute) to be measured to within 0.02 second.

Illumination of the stream. An accurately collimated beam of light was obtained by placing a point source at the focus of a 6" diameter concave mirror.

Measurement of the wavelength. The stream was aligned at right angles to the light beam, which was reduced by a shutter device to a narrow slit that illuminated the stream only. Under these conditions the waves function as cylindrical lenses producing astigmatic line images behind the jet (Fig. 1). These lines vary in width because of the effect of viscous forces on the stream, but the distances between their mid-points are equal to the corresponding wavelengths. The images were viewed on a translucent screen placed parallel with the stream. In order that the lines should be symmetrical about the stream it was necessary to have the major axis of the orifice parallel to the light beam. The wave images were recorded on a photographic plate which replaced the screen, and the wavelengths were determined to within 0.1 per cent. by measurements on the plate with a travelling microscope.

PLATE XVII.



Fig. 1. Line images produced from parallel light by the stationary waves on the jet stream. (x 1.3)



Fig. 2. Profile of orifice tip and jet stream showing wave formation. (x 12)

Measurement of stream radius. A photographic method was finally adopted which enabled a magnified image of the stream profile (Fig. 2) to be examined with a travelling microscope. One or more standard gauge wires (0.5 mm. diam.) were aligned close to and in the same plane as the stream, perpendicular to the light. For this purpose the slit limiting the width of the light beam was widened so that the stream and gauges were illuminated. The shadows cast were allowed to fall on an enlarging lens fitted in a half-plate camera placed behind and parallel to the stream. Using a red filter, exposures of a few seconds were necessary for satisfactory images on the plates used. After processing, the cross-section of the stream shadow was determined at intervals of 0.1 mm. near the wave inflexions, to obtain the maximum and minimum width of each. The true width was then calculated using the magnification factor obtained from measuring the gauge images. Stream radii—which varied with the orifice used—were within the range 0.01–0.025 cm. and were measurable to within $1\ \mu$ by this procedure.

Temperature Control. The laboratory temperature was maintained at a constant value ($\pm \frac{1}{2}^{\circ}\text{C.}$) within the range $18\text{--}21^{\circ}\text{C.}$ during a run, and all measurements were carried out at that temperature. The surface tension data were corrected to 20°C. using the accepted temperature coefficient value for water.

Purification of water. Demineralized water was used initially but this was later replaced by triple distilled water from a glass still lest undesirable side effects with surface active compounds (13) should occur.

Determination of the Surface Tension of Water. Only orifices that produced ten or more well defined, regular and symmetrical images were kept; these were further tested by photographing the line images, using any convenient flow rate, and measuring the linear separation of the wave images. Only those that gave constant wavelengths (within a few microns) after the first three or four waves were regarded as satisfactory.

Using an acceptable orifice, determinations were carried out at several flow rates. During a run at any one flow rate (lasting about 10 min.) it was necessary to take two replicate photographs of the wavelengths and one of the stream profile. For the latter the camera lens was arranged to view the selected section of the stream where the value of the wavelength was constant (e.g. 4–9th waves). At least three determinations of the flow rate were also made to establish its value and to confirm constancy.

The surface tension, γ_t at the temperature $t^{\circ}\text{C.}$, was then calculated from the Bohr formula which can be written in the simplified form:—

$$\gamma_t = \frac{4 A^2 \left(1 + 1.54 \frac{b^2}{r^2} \right)}{6 r \lambda^2 + 10 \pi^2 r^3}$$

where A = flow rate (ml./sec.)

λ = wavelength (cm.) at $t^{\circ}\text{C.}$

r = stream radius (cm.) $\approx \frac{1}{2} (r_{\text{max.}} + r_{\text{min.}})$

and $2r_{\text{max.}}$ = maximum cross-section of wave

$2r_{\text{min.}}$ = minimum cross-section of wave

and $b/r = \frac{r_{\text{max.}} - r_{\text{min.}}}{r_{\text{max.}} + r_{\text{min.}}}$

Results.

Of the large number of orifices made, four have been used to obtain tension data for water at several flow rates. The results are summarized in Table I.

TABLE I.
SURFACE TENSION OF WATER DETERMINED WITH FOUR ORIFICES.

Orifice	Flow rate (ml./sec.)	Wavelength (cm.)	Stream radius (cm.)	Surface Tension (dynes/cm.) (corr. to 20°C.)
L 2	0.5400	0.3575	0.0198	73.2
	0.5412	0.3587		73.1
L 3	0.5306	0.3499	0.0201	72.7
	0.7493	0.4993		72.8
H 3	0.4974	0.3207	0.0208	72.6
	0.6428	0.4205		72.9
	0.6485	0.4258		72.4
	0.8737	0.5822		72.8
	0.8787	0.5817		72.7
B 6	0.3129	0.2617	0.0127	73.5
	0.4060	0.3417		73.8
	0.4760	0.4028		73.4

Discussion.

Despite the practical importance of the behaviour of newly formed liquid surfaces, only few determinations of dynamic surface tensions have been reported in the literature. Although five methods have been developed for following the rapid aging of surfaces during the first 0.02 second after formation, the vibrating jet method as developed by Bohr is the only one having a sound theoretical and experimental basis. During the last fifty years, however, only Stocker (7), Rideal and Sutherland (9, 10) and Defay and Hommelen (11, 12) have succeeded in using the Bohr method for accurate measurements. Sutherland (9), using two orifices, obtained with pure water a constant value for the tension which was within 0.5 per cent. of the accepted static value. The tension values he obtained for a dilute aqueous solution of heptanol, however, differed by as much as 8 per cent. for the two orifices, and he concluded that accurate assessment of the rate of aging of solutions seemed to be dependent on the orifice used. Defay and Hommelen (11, 12), using seven selected orifices, obtained a different value (72.7—75.2 dynes/cm.) with each for the tension of pure water at 20°C. They applied a correction factor to the data from each orifice to convert the observed value to the classical value (72.8 dynes/cm.) and found that the same factors could be applied to the results obtained with aqueous solutions of heptanol.

The results in Table I show good agreement with the classical value for water. It has been found that the chief source of error is in the measurement of the stream radius, for although this can be made to within one micron, an error of this amount may approach one per cent. of the radius of a fine stream. An error in the stream radius produces the same percentage error in the final tension value, e.g. with orifice B6, Table I, the tension values that are little

more than one per cent. too high could well be due to a similar uncertainty in the stream radius value. Efforts are being made to increase the accuracy of the radius determination.

In spraying, wetting and other surface-dependent processes, liquid surfaces are either rapidly and extensively expanding in area, or being renewed continually. In such instances new air-liquid interfaces may be formed too quickly for the surface active compound to attain equilibrium concentration on the surface, with the result that it fails to reduce the surface energy effectively. Thus two formulations having the same equilibrium surface tension, and identical except for the surface active compound present, may behave quite differently in the field simply because one surface-active compound equilibrates more rapidly than the other. In such systems considerations of the static tensions of the surface active compounds may be completely misleading: information on the rates of aging at surfaces is likely to be more helpful in appraising the suitability of the compounds for particular processes. The results obtained for water in this work indicate that the method as developed is likely to prove valuable in the investigation of these dynamic systems. Whilst the determination of dynamic tensions has therefore a practical bearing in assessing the usefulness of a particular surface active compound, it is probable that a clearer understanding of the mechanism and kinetics of surface adsorption and desorption will also result from such studies.

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SPRAY APPLICATION PROBLEMS : LI. A PROTOTYPE LOW-PRESSURE TWIN-FLUID ATOMISER.

By T. E. COBBALD.

Introduction.

The size of air orifice required to provide a given power output from an air-assisted spraying machine varies inversely with the cube of the air speed. By employing relatively high air speeds, designers of such equipment are able to extract a high effective power output from components that are compact, light and easily adjusted or adapted to particular requirements. High air velocity in itself need not necessarily constitute a damage hazard to vegetation if the outlet orifices are suitably dimensioned and orientated.

The higher the air speed at the orifice, the more power there is available for the direct atomisation of the spray at that point. It has been found in practice that, if air speeds of 200 m.p.h. or more are employed, the ordinary type of liquid pressure nozzle may be dispensed with, since an adequate volume of the spray liquid can be satisfactorily atomised with very much simpler

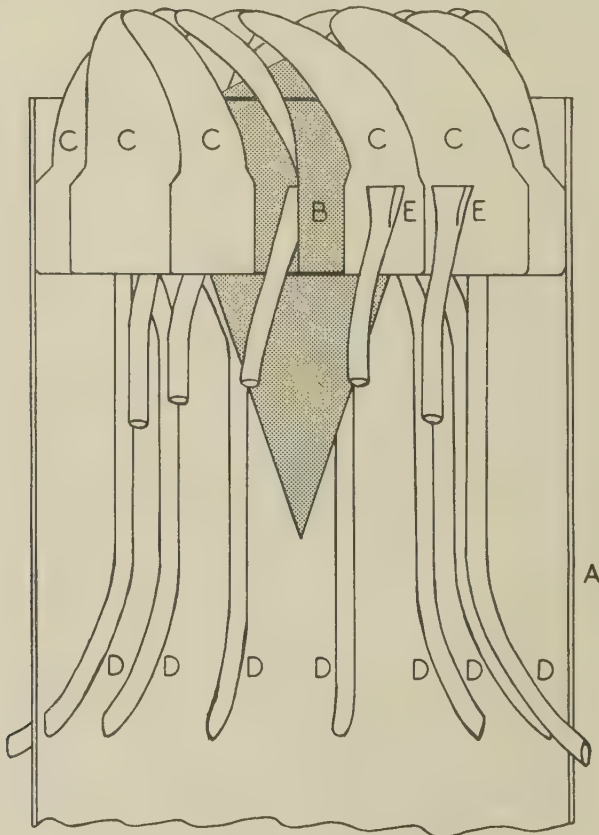


Fig. 1. Section of twin-fluid atomiser.



delivery equipment. If large volumes are to be atomised, however, it is important that the spray liquid should be given as high a surface/volume ratio as possible before being subjected to atomisation by the air stream. The nozzle described in this paper has been developed with this desideratum in mind.

Description (Fig. 1 and Plate XVIII).

The nozzle consists of a short length of pipe, 6" in diameter (A), in which is concentrically located a short 2" diameter cylindrical core (B), closed with cones at each end. Across the annular orifice thus remaining are twelve tongue-shaped vanes (CC), arranged radially. The basal sections of these vanes lie in planes coincident with the axis of the nozzle. The sides of the vanes are then sloped away from the pipe and core walls, and are also curved about axes normal to the nozzle axis.

Spray liquid is supplied separately to each vane through a $\frac{3}{16}$ " bore tube (D) that approaches the vane base, on the inner side, at an angle of approximately 15° , and is flared at the point of attachment (E) to give a rectangular orifice $\frac{3}{8}$ " by $\frac{1}{8}$ ". When the liquid is fed to the vane under moderate pressure (>10 lb. per sq. in.), it spreads out to form a film on the inner surface, continuing to fan out beyond the vane edge in the absence of a moving air stream. When air is passed through the nozzle it is partially deflected by the curved portions of the vanes, and the liquid films are thus exposed to a vigorous shearing action by the air streams around the vane edges. This continues the break-up of the spray liquid, which is carried away from the nozzle by the air stream. The vanes impart a considerable swirling motion to the air stream, which thus tends to diffuse at a wider angle than it would in the absence of a tangential velocity component.

With an air throughput of approximately 1,800 c.f.m. with a stagnation pressure of approximately 2 lb. per sq. in., up to 24 gallons of spray liquid per minute can be broken up. The liquid supply pressure for this throughput is 30 lb. per sq. in.

A critical assessment of droplet spectra will be described in a subsequent communication.

THE MICROBIOLOGY OF CIDER-MAKING.

By F. W. BEECH.

The present trend in cider-making is for production to be concentrated in a smaller number of factories, with a corresponding expansion in the scale of operations. Because of the increasing volume of each fermentation vessel, spoilage of a cider can now cause serious economic losses. Such losses can be avoided only if the process is under continuous microbiological control. Before such control measures can be developed it is essential that an extensive microbiological survey be made throughout the factory. Such a survey of the cider plant at the Research Station, and at a number of cider factories, has shown that while there are certain similarities in the micro-floras of all the factories, each factory seems to possess an additional micro-flora peculiar to itself. It is this specialised "factory flora" that is so important; if it consists of undesirable organisms the cider produced will always be tainted or difficult to clarify.

An account is given of the changes in the composition of the micro-flora in the cider house of the Research Station at all stages of cider-making, starting with the fruit (1, 2, 3, 4).

Fruit.

Yeasts are transferred to the open flower buds by bees, the yeasts having overwintered in the hives (5). Most of these yeasts form films or pseudomycelia and ferment only feebly. As the fruitlet develops, the yeasts, trapped originally at the base of the anthers and the stigmas, pass into the axial sac and thus appear in the core of the mature apple. At the same time the skin of the fruit becomes infected with yeasts carried up from the soil by crawling insects or by winds. Most of these yeasts form starch or coloured pigments and again ferment little if at all, but a few of the yeasts on the skin consist of strongly fermenting yeasts of the genus *Saccharomyces*. The soil in orchards, in vineyards, and under beehives, always contains a higher proportion of yeasts than soils of other localities (5). Should the skin of the fruit become damaged by abrasion, or by insects or birds, the flesh is invaded and softened by moulds. The exuded juice encourages the growth of the strongly fermenting yeasts, which develop extensively in the damaged flesh under the skin (6).

When the fruit falls or is shaken from the trees it becomes contaminated with lactic and acetic acid bacteria (7), especially the latter if fruit flies are present in any numbers. Prolonged storage in sacks or badly designed fruit silos causes juice to be expressed from the damaged fruit, and the whole population of moulds, yeasts and bacteria increases correspondingly. Thus the juice can be tainted before the fruit is pressed. Washing the fruit removes a great deal of the infected juice, but more careful treatment at an earlier stage would have prevented the loss of juice quality and of much juice sugar.

Juice.

The yeast flora of a juice from the pilot-plant at the Research Station, in which freshly washed cloths and racks are used for each pressing, is very similar to that of the fruit from which it is made, i.e. it consists mainly of poorly-fermenting yeasts and contains very few strongly-fermenting yeasts.

However, when other batches of the same fruit are processed in commercial plant, the juice contains not only a much greater percentage of *Saccharomyces* spp., but the total numbers of yeasts and bacteria are much greater. Typical results, obtained by counting living organisms per ml. of juice, are as follows :—

		<i>Yeasts</i>	<i>Bacteria</i>
Juice 1	{ Pilot-Plant	610,000	135,000
	{ Factory A	1,370,000	185,000
Juice 2	{ Pilot-Plant	190,000	132,000
	{ Factory B	16,100,000	280,000

The juices are heavily contaminated with yeasts in both factories and, to a lesser extent, with bacteria. In both factories the cloths are washed very infrequently and the press racks not at all. This is obviously an unsatisfactory procedure since it is at this stage that the greatest proportion of the factory flora is introduced.

Pre-Fermentation Treatments.

Apart from the organisms introduced as contaminants from the equipment, the fresh juices themselves usually contain organisms that may have deleterious effects. Fortunately the traditional system of cider-making, whereby ciders are fermented and stored anaerobically, has provided conditions unfavourable to many undesirable organisms. Pure yeast fermentation would provide a more logical but more expensive method. It would involve a rapid depectinisation of the juice, clarification in a self-desludging centrifuge, and flash pasteurisation into sterilised stainless steel or lined-steel pressure tanks, the cider then being yeasted from a pure culture plant. With proper laboratory supervision this system would yield ciders of clean flavour. The reduction in the numbers of living organisms per ml. of juice during this sequence of operations is shown below :—

	<i>Yeasts</i>	<i>Bacteria</i>
Untreated juice	625,000	510,000
Depectinised juice	325,000	520,000
Filtered juice	320	985
Flash pasteurised juice	0	0

The system cannot be adopted where only wooden fermentation tanks are used in the factory (8). It would be even more dangerous to try to ferment untreated juice in pressure tanks, since any lactic acid bacteria present would develop rapidly under these conditions. Simply centrifuging untreated juice is also dangerous because the number of bacteria is proportionately increased, as shown below from counts on living organisms per ml. of juice.

		<i>Yeasts</i>	<i>Bacteria</i>
Juice 3	{ Untreated	400,000	65,000
	{ Centrifuged	200,000	550,000
Juice 4	{ Untreated	5,900,000	N.D.
	{ Centrifuged	2,155,000	55,000

N.D. = Not detected.

In the traditional type of cider factory, using wooden vats, the addition of sulphur dioxide to the juice offers a practical form of control. Certain

points must be borne in mind. The sulphur dioxide does not exert its maximum inhibitory action until at least four to six hours after being added to the juice (3). Consequently freshly sulphited juices should not be yeasted or mixed with fermenting juices until after this holding period. Further, the inhibitory action of sulphur dioxide on yeasts and bacteria varies with the pH of the juice (9). Sulphur dioxide is extremely effective at pH 3.0, whereas at least ten times as much is required at pH 4.0 to produce the same effect on the flora. An example of the effect of 150 p.p.m. sulphur dioxide on the viable organisms per ml. of juices of different pH is given below.

<i>pH</i>	<i>Juice</i>	<i>Yeasts</i>	<i>Bacteria</i>
3.43	Untreated	3,250	9,150
	Sulphited	25	50
4.0	Untreated	685,000	510,000
	Sulphited	41,500	20,000

Thus it is better to vary the amount of sulphur dioxide according to the pH of the juice. Adequate amounts would be : 50 p.p.m. for pH 3.0 to 3.3, 100 p.p.m. for pH 3.4 to 3.9 and 150 p.p.m. for pH 4.0 and over. These levels are satisfactory for pure juice ciders, but if standard alcohol ciders are to be prepared at a later date, by dilution and re-fermentation, then 150 or even 200 p.p.m. SO_2 should be added, irrespective of the pH. Only fermenting yeasts and sickness bacteria (if present) remain after the addition of sulphur dioxide. Non-fermenting yeasts and many bacteria are killed ; moulds are greatly reduced in numbers.

If sulphur dioxide is used indiscriminately there is a danger that a population of sulphite-resistant bacteria, especially acetic acid bacteria, will be built up in the factory. The sulphur dioxide should be metered into the juice as it is pumped from the press ; it is essential that this juice be allowed to stand several hours before mixing with fermenting cider. The sulphiting treatment will be even more effective if the micro-flora of the untreated juice is kept as low as possible ; thus, press cloths and racks must be washed daily. An undesirable micro-flora can also build up readily in wooden vats ; these vessels should be scrupulously cleaned each time they are emptied and, as circumstances permit, replaced by vessels with impervious surfaces.

Changes in the Micro-Flora during Fermentation.

In the fermentation of untreated juice the film-yeasts and poorly-fermenting yeasts die out almost entirely during the first five degrees loss of specific gravity. They do not disappear completely and may multiply later, especially in badly stored ciders. Non-sporulating apiculate or lemon-shaped yeasts show a temporary increase in numbers over this initial period and finally die out after a loss of about 35° of specific gravity. The most startling change in the yeast flora is the enormous increase in the number of strongly fermenting yeasts : these consist of several species of the genus *Saccharomyces*, one or more of which may be derived from the fruit and the remainder from the processing plant. This increase starts after only one or two degrees drop in specific gravity and reaches a maximum after a loss of 15°. If the nutritional status of the juice is originally high, the number of living yeasts does not decrease appreciably until the end of the fermentation : if low, then the

numbers decrease continuously throughout the remainder of the fermentation. These changes in the micro-flora during fermentation are shown in Table I for a low nitrogen juice obtained from a commercial plant.

TABLE I.
CHANGES IN THE NUMBERS OF VIABLE YEASTS DURING THE FERMENTATION OF AN
UNTREATED LOW NITROGEN JUICE.
Viable organisms per ml.

Specific gravity	Loss of specific gravity	Poorly fermenting yeasts	<i>Saccharomyces</i> spp.	Apiculate yeasts	Unknown Yeast I
1,060	0°	232,100	643,000	179,400	N.D.
1,055	5°	N.D.	11,650,000	880,000	N.D.
1,045	15°	N.D.	22,400,000	250,000	N.D.
1,035	25°	N.D.	12,350,000	180,000	1,150,000
1,025	35°	N.D.	7,280,000	100,000	2,750,000
1,005	55°	N.D.	5,000,000	N.D.	900,000

N.D. = Not detected.

In the fermentation of the same juice after treatment with sulphur dioxide the yeast population is smaller at first : nearly all yeasts except the *Saccharomyces* spp. die out within 5° loss in gravity. The *Saccharomyces* spp. reach a maximum at the same point as in the non-sulphited juice, but their number is much greater, owing no doubt to lack of competition for nutrients from other yeasts. In both sulphited and non-sulphited juices, another strongly fermenting yeast slowly reaches a maximum towards the end of the fermentation ; the role and identity of this yeast have not yet been determined.

The development of bacteria is conditioned by the initial population, by the nutritional status and pH of the juice, and by the addition of sulphur dioxide. With a non-sulphited low-acid (high pH) juice, made from damaged fruit grown on heavily manured land, it is possible for bacteria greatly to outnumber the yeasts and for a malo-lactic fermentation to take place before yeast fermentation commences. At the other extreme, in very acid (low pH) sulphited juice from sound fruit grown in starved orchards, bacteria may not appear at all, even when the cider is stored on its lees for a considerable period. The general effect of sulphur dioxide on the activity of the micro-flora at different pH levels was determined by Carr and Whiting (10) ; their amended results have been summarized below :—

Below pH 3.4	{	Untreated :	Malo-lactic fermentation.
	{	Sulphited :	No marked change in acidity.
pH 3.4 to 4.5		Untreated :	The malic acid derived from the juice and from yeast activity is subsequently reduced by malo-lactic or malo-succinic change.
pH 3.4 to 4.0		Sulphited :	Acidity increased due to the formation of malic and pyruvic acids by yeasts.
pH 4.0 and over		Sulphited :	As for untreated juices in the same pH range.

Succinic acid is formed by yeasts in the fermentation of both untreated and sulphited juices, the amount increasing with increasing pH.

A characteristic feature of the fermentation of sulphited juices is the increase in acidity caused by the formation of malic and pyruvic acids. This is of great practical importance, especially if the strength of the cider has to be reduced before bottling. The formation of malic acid is not confined to sulphited juices : it is also produced in non-sulphited juices but is subsequently lost during the malo-lactic fermentation. This loss of malic acid is undesirable in West Country cider-making, where fruit of medium acidity is used, since it must be replaced at some expense with citric acid.

In the Eastern Counties, where a more acid fruit is used, the situation is reversed and the malo-lactic fermentation is encouraged. Nevertheless, the addition of a small quantity of sulphur dioxide to the juice is desirable to encourage a cleaner yeast population during fermentation. It has been found that the micro-floras of dry ciders made from untreated and sulphited juice consist of approximately the same number of yeasts, but the cider from untreated juice contains a large proportion of undesirable apiculate yeasts, whereas in the sulphited cider all the yeasts are *Saccharomyces* spp.

Processing of Dry Ciders and Storage.

To prevent deterioration in the quality of the dry cider it is necessary not only to exclude air but also to avoid any processing treatment that allows the development of other spoilage organisms.

Ciders made from untreated high pH juices spoil rapidly if left on their lees : the yeasts autolyse and encourage the development of lactic acid bacteria. Racking or centrifuging without further clarification is also dangerous, since it removes the majority of the yeasts but leaves the number of bacteria virtually unchanged. Filtration following racking or centrifuging removes most of these bacteria, as shown by the viable counts, 1 ml. given below for a high pH juice, but it does not restore the quality of the cider.

	<i>Dry Cider from Untreated juice.</i>		<i>Dry Cider from Sulphited juice.</i>	
	<i>Yeasts</i>	<i>Bacteria</i>	<i>Yeasts</i>	<i>Bacteria</i>
Mixed	600,000	10,350,000	27,000,000	+
After centrifuging	40	4,300,000	<1,000	N.D.
After centrifuging and filtering	N.D.	8,650	N.D.	N.D.
N.D.=Not detected. +=present, but in very small numbers.				

In ciders from low pH juices these problems do not arise to the same extent since bacterial action is inhibited by the high acidity.

Bacterial spoilage is not likely to arise in high pH ciders if they have been made from sulphited juices. It is still advisable to filter the ciders and store at as low a temperature as possible, otherwise the malo-lactic fermentation will occur eventually. Blending the dry ciders to an acidity of 0.5 to 0.6% before storage will also delay bacterial action. Even with these precautions, factories using wooden fermentation and storage vessels will find it very difficult to prevent malo-lactic fermentation during prolonged storage.

The effect of lack of cleanliness in factories using wooden vats is shown below from the changes in the number of viable organisms per ml. of cider in its passage from the storage tank to the trade cask.

	<i>Yeasts</i>	<i>Bacteria</i>
Cider before centrifuging	10,800	7,500
Direct from the centrifuge	25	5,000
End of hose from centrifuge	120	5,000
Wooden tank of centrifuged cider	1,500	5,000
Final sweetened cider in trade cask	12,500	N.E.

N.E. = Not estimated.

Contamination from the equipment is such that the clarifying treatment is ineffective, except for the removal of dead yeasts. It is not surprising that ciders of this kind often ferment in the trade casks during distribution and sale.

Discussion.

Several conclusions can be drawn from the foregoing microbiological survey of cider-making practices. Perhaps the most important is that an undesirable population of yeasts and bacteria can be built up in the cider factory. This development of a factory flora occurs in other factories where fermented food or pickled products are manufactured. The problem is aggravated by lack of hygiene, especially failure to wash press cloths and racks at frequent intervals, and by the use of wooden vats and old rubber hoses. Keeping the factory and equipment scrupulously clean (11, 12) would eliminate much of the undesirable micro-flora. Wooden vats, because they are relatively cheap compared with vats made of other materials, will need to be used so long as cider-makers ferment the whole of one season's make, while storing at least part of the previous season's cider. If, instead, cider-makers concentrated the greater part of the season's juice, they would need to ferment relatively smaller volumes at regular intervals throughout the year. It would then be possible to use the more expensive, but much more satisfactory, stainless steel or lined-metal vessels of reasonable capacity. The use of apple concentrates with vessels of this type has a number of advantages. The cider-maker could prevent bacterial development by using a pure culture fermentation of the flash pasteurised juices; by careful control of the nitrogenous and sugar contents of the diluted concentrates, a cider with a more uniform period of fermentation and a standard alcohol content could be produced. This system is similar to that operated successfully in breweries and is already being used for making some high grade ciders and perries.

Summary.

The results of a microbiological survey of a number of cider factories are described. The effects of different cider-making practices on the micro-flora of the juice or cider are discussed. An outline is given of a method whereby microbial spoilage may be prevented.

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ACETIC ACID BACTERIA IN CIDERS.

By J. G. CARR.

Introduction.

Acetification, the earliest known and most easily recognized disorder of cider, arises from the bacterial oxidation of the ethyl alcohol produced by fermentation and imparts an unpleasant vinegary flavour and aroma. This taint was frequently encountered, and until recent years was almost expected, in many farm ciders. Changes in the tastes of the consumers and the increased commercialization of cider-making have led to the virtual eradication of the disorder, largely by improvements in methods of storage and the use of sulphur dioxide during fermentation. The acetification of cider has never been extensively studied and the only information on acetic acid bacteria in English ciders is limited to the description of one named species (1). Outbreaks of acetification still occur from time to time, and a further study of the problem has suggested that the factors concerned in the disorder have been oversimplified.

Work done elsewhere (2) has shown that the acetic acid bacteria can be divided into two categories. The first has the name *Acetobacter*; its members are distinguished by having peritrichous flagella when motile (3) and by their ability to oxidize ethyl alcohol not only to acetic acid but to continue the oxidation to carbon dioxide and water. These are the true vinegar bacteria, and organisms of this type are found in the generators used for commercial vinegar production. This group contains the organism most familiar to cider-makers (*Acetobacter xylinum*), best known because of its ability to produce cellulose which forms a strong and buoyant mat at the liquid-air interface, thus enabling it to obtain the oxygen essential for growth and acetification. Other members of the group form more delicate surface films which readily disintegrate and sink when agitated, thus becoming deprived of oxygen.

The second group, for which the name *Acetomonas* has been proposed (2), has never been described previously in cider although certain of its members are known to cause ropiness in beer; they are distinguished from *Acetobacter* species by their inability to oxidize ethyl alcohol further than acetic acid and by possessing polar flagella if motile.

Since both groups of organisms are aerobic, the process of fermentation, which is essentially anaerobic, might be expected to suppress them if not to kill them completely. The present investigation was therefore made to follow the occurrence and growth of the acetic acid bacteria through all the stages of cider-making.

Experimental.

To study the organisms present on the fruit, a sample of Yarlington Mill apples was taken aseptically at harvest time by cutting their stems and allowing them to drop into a sterile jar. A further fruit sample, containing bruised apples as well as others contaminated with grass and soil, was collected beneath the tree. Individual apples of the sample taken from the tree were swabbed with sterile water; the bulked washings plus the homogenized swabs were centrifuged and the deposit obtained was plated on two types of media that permitted the growth of acetic acid bacteria or yeasts (4). The apples taken from the ground were treated similarly.

The crop was harvested from the chosen tree in the usual manner, milled and pressed and the resultant juice used to fill two sterile 3-gallon jars two-thirds full, thus exposing a large surface area of liquid. One of the jars (aerobic fermentation) was plugged to allow the free entry of air and exit of carbon dioxide while preventing the entry of extraneous micro-organisms. In the other jar (anaerobic fermentation) the air space above the juice was constantly swept out by a stream of sterile carbon dioxide. These two treatments were maintained throughout fermentation and storage of both ciders. Periodically samples were removed aseptically from each fermentation and serially diluted on selective media (4) to obtain separate counts of yeasts and acetic acid bacteria.

Results.

The distribution of organisms on the fruit samples was as follows: *Acetobacter mesoxydans* and *Acetomonas suboxydans* plus yeasts and moulds were isolated from the fruit taken from the ground, while only yeasts and moulds were found on the fruit taken from the tree.

Table I indicates the relative numbers of yeasts and acetic acid bacteria present in the pressed juice and in the two jars before active fermentation had started; during the period of maximum yeast activity; and later during storage. Although the juices in the two jars were initially the same in their chemical composition and microbial flora, the oxygen level had a marked effect upon the development of the yeasts during fermentation. The juice in the anaerobic jar was slower to start fermenting but fermentation later proceeded so rapidly that after twenty days the specific gravity was eleven degrees lower than that of the aerobic fermentation. This could be attributed to the denser yeast population—26,900,000 organisms per ml. at twenty days compared with only 5,000,000 organisms per ml. in the aerobic fermentation.

TABLE I.

RELATIVE NUMBERS OF YEASTS AND ACETIC ACID BACTERIA IN AEROBIC AND ANAEROBIC FERMENTATIONS.

Day of sampling	Anaerobic fermentation							
	0	7	14	20	42	67	82	96
Specific gravity	1050	1047	1035	1022	1002	1002	1002	1002
Yeast count $\times 10^{-6}$ cells per ml.	1.39	3.5	42.4	269	41	4.6	12.3	1.2
Bacterial count $\times 10^{-6}$ cells per ml.	0.48	2.4	0.36	0.23	0.001	0.046	0.61	0.2
	Aerobic fermentation							
	1050	1046	1043	1033	1008	1002	1002	1002
Specific gravity	1050	1046	1043	1033	1008	1002	1002	1002
Yeast count $\times 10^{-6}$ cells per ml. ..	1.39	1.41	3.7	50	19.3	40	141	16.9
Bacterial count $\times 10^{-6}$ cells per ml.	0.48	2.1	5	4.7	0.16	0.18	3.1	1.2

In both jars fairly rapid development of the bacteria occurred before fermentation had actively begun. However, under the conditions of intense yeast activity in the anaerobic jar between the seventh and twentieth days, the bacterial numbers rapidly fell and remained low until the fermentation had ceased. In contrast, the first bacterial growth peak in the aerobic jar was more sustained and of greater magnitude, and even at the time of maximum yeast growth (twenty days), the bacterial numbers were not very different from those recorded six days previously. It was only at the end of the most active phase of yeast growth that a marked decline in bacterial numbers occurred in this jar. During storage, the acetic acid bacteria with free access to the air reached five times the concentration of those under an atmosphere of carbon dioxide. It is of interest to note that the development of non-fermenting aerobic yeasts, coinciding with the bacterial growth, was also suppressed in the presence of carbon dioxide.

Representatives of both groups of acetic acid bacteria were isolated during this experiment. The two species which constitute the *Acetomonas* group, namely *Acetomonas suboxydans* and *Acetomonas melanogena*, were found at the beginning of the fermentation and persisted in both ciders until the later stages of storage. In contrast, the *Acetobacter* species, represented initially by *Acetobacter mesoxydans*, disappeared during the period of most intensive yeast activity, to reappear accompanied by *Acetobacter xylinum* and *Acetobacter rancens* when fermentation had virtually ceased.

The multiplicity of species found in the ciders suggests that these bacteria were introduced from sources other than the fruit: no precautions had been taken to avoid the introduction of extraneous micro-organisms during the milling and pressing operations. Alternatively, the two additional species of the group *Acetobacter* may have been derived from the original species *Acetobacter mesoxydans* by mutation, since it is known that organisms of this group can readily change from one species to another spontaneously (5).

Discussion.

Two main facts, hitherto unknown, have emerged from this experiment. First, the soil and plant debris collected with the fruit is a rich source of acetic acid bacteria. The traditional method of harvesting cider apples by shaking them from the tree must therefore contribute substantially to the initial number of acetic acid bacteria occurring in a cider. Secondly, the fact that these aerobic organisms can not only survive the rigours of fermentation but multiply during certain stages is new and surprising. One point that needs further investigation is how these bacteria survive and what the nature of their metabolism is during the periods when oxygen is absent.

In comparison with the number of yeasts present during fermentation, the population of acetic acid bacteria is very small. Nevertheless, the presence of even a small population of these organisms in a cider is a potential danger to its quality, particularly if conditions arise to favour their development in the later stages of cider-making. This risk can, however, be minimized by efficient washing of the fruit before milling and pressing, and by the use of sulphur dioxide in the juice (6).

Summary.

- (1) Acetic acid bacteria of the groups *Acetobacter* and *Acetomonas* have been isolated from apples contaminated with soil and plant debris, but not from fruit taken directly from the tree.
- (2) These organisms are not only able to survive at a low level during fermentation but to increase during fermentation and also, to a small extent, in a cider kept under a constant atmosphere of carbon dioxide.
- (3) The bacteria that survive fermentation most easily are of the group *Acetomonas*. Those of the group *Acetobacter* are reduced to an undetectable level during the most intensive phase of fermentation.

Acknowledgment.

I am indebted to Miss J. Bird for valuable assistance during this work.

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CHANGES IN SULPHUR DIOXIDE CONTENT DURING FERMENTATION OF CIDER.

By L. F. BURROUGHS.

The addition of sulphur dioxide to apple juice before fermentation is now common practice in cider-making : by this means most of the undesirable yeasts and bacteria are inhibited and the fermentation is dominated by the main fermenting yeasts. The resulting cider is fresher, more fruity in flavour and less liable to lose acidity by malo-lactic fermentation during storage (1).

When SO_2 is added to fruit juices it is generally assumed that part is oxidised by dissolved oxygen to sulphate and that part combines with aldehydes and other compounds in the juice. The rest of the SO_2 in solution remains free in the form of undissociated sulphurous acid and the sulphite and bisulphite ions, the equilibrium between these species depending on the pH. Only the free SO_2 is considered to be effective against micro-organisms (2). Little is known, however, of the detail of these processes in cider-making or even in wine-making, where sulphited fermentations have been used for many years. Joslyn & Braverman (2), in a comprehensive review of the preservation of fruit and vegetable products with SO_2 , state : " In spite of extensive investigations on wine making . . . complete analyses of the distribution of SO_2 in wines and of changes in the sulphur compounds during storage are not available." Mills & Wiegand (3) examined the total- SO_2 content of 210 wines of various types before and after storage in bottle for six months. They found that losses of SO_2 were generally proportional to the initial SO_2 content ; very high sugar content gave some protection against loss of SO_2 but alcohol content had no effect. Paul (4) studied the effect of SO_2 content of juices (black currant, grape and apple) on the amount of acetaldehyde produced during fermentation and his results have some bearing on the present problem.

In view of the current importance of the use of SO_2 in cider-making, the experiments described in this paper were designed to follow the changes in SO_2 content during fermentation, bottling and storage.

Experimental.

Two $3\frac{1}{4}$ gallon jars of juice (3 volumes Sweet Alford, 1 volume Court Royal) each received 55 ml. of approximately 5% SO_2 solution. One jar (A) was fitted with two tubes so that samples could be dispensed by top-pressure from a CO_2 cylinder. Samples were taken at suitable intervals until fermentation commenced and were analysed for total- and free- SO_2 content.

The second jar (B) was fitted with two absorption U-tubes, each containing 10 ml. " 10 volume " H_2O_2 neutralised to bromophenol blue. In this way any SO_2 carried over in the CO_2 evolved during fermentation would be oxidised to H_2SO_4 and could be titrated with standard NaOH.

When the cider in jar B had fermented to dryness it was thoroughly mixed to resuspend the yeast, and a sample was removed for analysis. The remaining cider was centrifuged (Alpha Laval), filtered through clarifying sheets (Carlson K5) and stored overnight at 0°C. Half of the cider was sweetened to S.G. 1.020 by adding sugar, and both the sweet and dry ciders

were lightly carbonated, bottled in half-pint, crown-cork bottles and pasteurised (raised to 68°C. in 20 minutes, held for 20 minutes and then cooled). The total-SO₂ content of the cider was determined after each of these operations and during storage in bottle at cellar temperature.

Total SO₂ was determined by distilling 50 ml. cider with 200 ml. *N* HCl and titrating the distillate continuously with 0.05 *N* iodine to maintain the starch-blue end point (5).

Free SO₂ was determined by titrating 50 ml. cider plus 5 ml. 25% (by volume) H₂SO₄ with 0.02 *N* iodine using 2 ml. 1% starch solution as indicator.

The *Volatile Aldehyde* content of cider is assumed, for present purposes, to consist of acetaldehyde.

Total acetaldehyde was determined (6) by neutralizing 25 ml. cider with NaOH and distilling with 25 ml. pH 9 buffer (2.5% sodium borate in 0.025 *N* H₂SO₄); 25 ml. of distillate was collected in a flask containing 25 ml. pH 7 buffer (15 g. Na₂HPO₄, 12H₂O and 3.35 g. KH₂PO₄ per litre) and 5 ml. 2% NaHSO₃. After acidifying with 5 ml. 25% v/v HCl, the excess SO₂ was discharged with iodine using 1% starch solution as indicator. The solution was then titrated with alkaline borate (3% H₃BO₃ in *N* NaOH) to a phenolphthalein end point, and the SO₂ liberated from the aldehyde bisulphite was *immediately* titrated with 0.01 *N* iodine (1 ml.=0.22 mg. acetaldehyde).

Free acetaldehyde can be recovered quantitatively from cider at its natural pH by vacuum distillation at 30°C., and under these conditions acetaldehyde bisulphite is not appreciably decomposed.

Cider (25 ml.) was distilled in a Quickfit micro-Kjeldahl distillation unit at 30 (±1)°C. and 15-20 mm. pressure with a small air-leak, via a short condenser into an ice-cooled U-tube containing 5 ml. of buffered NaHSO₃ (5 vol. pH 7 phosphate buffer—see above—plus 2 vol. 2% NaHSO₃); about 5 ml. of condensate was collected in 15 minutes. The solution from the U-tube was washed into a flask and its acetaldehyde bisulphite content estimated as described above for total aldehyde.

Results.

The analysis of the original juice was: S.G.=1.054; acidity=0.18% as malic acid; pH=4.04; tannin=0.12%; soluble N=10.0 mg./100 ml.

SO₂ changes during fermentation.

The total- and free-SO₂ contents of the juice in Jar A during the first four days are shown below; visible yeast growth and evolution of CO₂ were observed on the 4th day.

Sulphur dioxide (p.p.m.)

<i>Time</i>	<i>Total</i>	<i>Free</i>
5 min.*	174	—
20 "	—	88
30 "	—	84
2 hours	173	72
4 "	166	68
22 "	170	64
3 days	162	62
4 "	163	6

*i.e. 5 min. after the addition of SO₂.

The total SO_2 content showed only a small decrease during the four days and at the end of the fermentation (16th day, S.G.1.002) was still 163 p.p.m. On the other hand, the free SO_2 content dropped to 88 p.p.m. in the first 20 minutes and then decreased very slowly up to the third day. With the onset of fermentation the free SO_2 was completely fixed, presumably by the acetaldehyde produced as a normal intermediate metabolite. The 6 p.p.m. recorded on the 4th day was probably no more than a blank titration due to reducing substances other than SO_2 .

Aldehyde content of the dry cider.

These determinations were made on dry cider (K5 filtered) from Jar B, since this fermentation had not been disturbed by removal of samples. This cider contained 161 p.p.m. total SO_2 , of which not more than 9 p.p.m. was free. It was therefore of interest to see how much of the combined SO_2 could be accounted for by acetaldehyde.

The total- and free-aldehyde contents of the cider were found to be 79 and 7 p.p.m. respectively, so that the combined acetaldehyde content was 72 p.p.m. If it is assumed that all the combined acetaldehyde was in the form of acetaldehyde bisulphite, this was equivalent to 105 p.p.m. SO_2 .

A supplementary experiment showed that fermentation in the presence of SO_2 increases the acetaldehyde content of a cider. A Court Royal juice, with and without the addition of 150 p.p.m. SO_2 , was fermented to dryness by its natural yeasts. The free- and total-aldehyde contents are shown below.

Acetaldehyde (p.p.m.)

	<i>Free</i>	<i>Total</i>
Juice	2.5	5.5
Cider (no SO_2)	10.0	19.2
Cider (150 p.p.m. SO_2)	6.0	63.0

Possible losses of SO_2 during fermentation.

(a) *In the CO_2 stream.* The H_2O_2 in the absorption tubes on Jar B was titrated on the 4th day (when fermentation started) and again on the 16th day at the end of fermentation. No significant amount of SO_2 was detected as sulphate on either occasion. This was not unexpected, since during the first four days when the juice still contained free SO_2 there was no CO_2 evolved to carry it away, while during the active fermentation there was no free SO_2 present in the cider. Nevertheless, this check was necessary to show that there was no mechanical loss of SO_2 from the system.

(b) *By yeast absorption.* On the 16th day a sample of dry cider (S.G.1.002) from Jar B, including the suspended yeast, contained 160 p.p.m. SO_2 . When a 750 ml. portion of this cider was centrifuged, the supernatant contained 159 p.p.m. total SO_2 (9 p.p.m. free); when the yeast deposit was suspended in 55 ml. of the cider, the suspension contained 137 p.p.m. SO_2 . There was, therefore, no absorption of SO_2 by the yeasts; the lower SO_2 content of the yeast suspension was probably due to the volume occupied by the yeast cells.

SO₂ changes during bottling, pasteurising and storing.

The cider remaining in Jar B was bottled, the total SO₂ content being determined after each operation and after pasteurisation and storage.

Centrifuged	..	..	..	..	159	p.p.m. SO ₂
Filtered	..	..	..	..	161	" "
Stored overnight at 0°C.	..	..	..	..	157	" "
Sweetened	..	..	..	..	151	" "
Carbonated and bottled	..	(sweet)	..	..	150	" "
"	"	(dry)	..	..	156	" "
Pasteurised	..	..	(sweet)	..	143	" "
"	..	..	(dry)	..	147	" "
Stored 4 weeks	..	..	(sweet)	..	133	" "
"	"	"	(dry)	..	137	" "
Stored 14 months	..	..	(sweet)	..	120	" "
"	"	"	(dry)	..	125	" "

The lower figures for the sweetened cider were due to the increase in volume (3.4%) on adding sugar.

Discussion.

Since it was shown that no SO₂ escaped from the jars during fermentation and since the dry cider contained no free SO₂ that could be lost by volatilisation, the recorded losses of SO₂ are tentatively attributed to oxidation, presumably to sulphate. Such oxidation is known to occur in wines (7). Attempts to determine small differences in sulphate content of the cider in this experiment were unsuccessful and until such determinations can be made the hypothesis of oxidation to sulphate remains the simplest explanation of the disappearance of total SO₂, but the possibility of other degradations of SO₂ is not excluded.

In this experiment the changes in total- and free-SO₂ content during fermentation were generally similar to those found by Paul (4) in grape and apple juices. He also recorded the rapid fixation of free SO₂ added to the juice. Apple juices normally contain in the region of 10 p.p.m. acetaldehyde and this would combine rapidly with about 15 p.p.m. SO₂. Traces of other aldehydes and ketones in the juice may also react rapidly with SO₂, but the main SO₂-combining substance is undoubtedly glucose, which in this low acid juice (pH 4) would also react fairly quickly (8). Fixation of SO₂ by juice constituents appeared to be complete in two hours and the subsequent very slow decrease in free SO₂ (10 p.p.m. in three days) was accompanied by a similar loss of total SO₂, attributable to oxidation.

The dry cider contained 152 p.p.m. combined SO₂ and 69% of this (105 p.p.m.) could be attributed to acetaldehyde bisulphite. These results for free and total aldehyde were similar to those of Paul (4) as also were the aldehyde contents of the Court Royal ciders fermented with and without SO₂.

The losses of SO₂ during the various operations of bottling were very small, probably because the dry cider contained little or no free SO₂. Direct titration of the free SO₂ in the centrifuged cider immediately before bottling gave a value of 9 p.p.m., approximating to the blank due to other reducing substances.

Greater losses of SO₂ were found after pasteurising and during storage. If these losses were due to oxidation this must have been caused by the oxygen

content (not determined) of the cider before bottling and by air in the head-space of the bottle. The average volumes of cider and head-space were 280 ml. and 15 ml. respectively. Assuming the head-space contained air at normal pressure this would be sufficient to oxidise 50 p.p.m. SO_2 in 280 ml. of cider. It is, however, probable that the head-space also contained some CO_2 from the lightly carbonated cider.

The overall loss of SO_2 (31 p.p.m.) during pasteurisation and storage for fourteen months was appreciably less than the average loss of 54 p.p.m. found by Mills & Wiegand (3) in a group of 102 wines with an average initial SO_2 content of 150 p.p.m. after storage for six months. Presumably their bottles were sealed with corks and this may have allowed some entry of air. This would not occur with the crown-corked bottles of cider, and loss of SO_2 may have been limited here by the original oxygen content.

In order to establish a complete balance sheet for SO_2 during fermentation, bottling and storage of cider, more detailed analysis is required, including the determination of sulphur compounds other than SO_2 . The situation is undoubtedly complex and other conversions of SO_2 must be considered as well as oxidation to sulphate; for example, reduction of SO_2 to sulphide and mercaptans has been reported for wines (7). The present results, however, indicate the magnitude of the losses of SO_2 to be expected in cider-making operations and the manner in which free SO_2 combines with other constituents of the juice or cider.

Summary.

- (1) An apple juice containing 174 p.p.m. total SO_2 lost only 11 p.p.m. during fermentation to cider.
- (2) Rapid fixation of SO_2 by juice constituents reduced the free SO_2 content to 88 p.p.m. within 20 minutes; free SO_2 disappeared completely with the onset of fermentation.
- (3) No SO_2 was lost in the CO_2 evolved during fermentation and no SO_2 was absorbed by the yeasts.
- (4) In the dry ciders, 69% of the combined SO_2 was present as acetaldehyde bisulphite.
- (5) Only about 4 p.p.m. SO_2 was lost during centrifuging, filtering, carbonating and bottling, but 9 p.p.m. was lost in pasteurising.
- (6) During storage for 14 months, 22 p.p.m. SO_2 was lost, the final SO_2 content being 125 p.p.m.

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THE NON-VOLATILE ORGANIC ACIDS OF CIDER-APPLE JUICES : CHANGES DURING GROWTH AND STORAGE OF THE FRUIT.

By G. C. WHITING and R. A. COGGINS.

Introduction.

Previous work has shown the presence in cider-apple juices of a number of non-volatile organic acids (1). In all the varieties examined malic acid was predominant, with quinic acid present in next greatest amount; citric, galacturonic, chlorogenic, caffeic, shikimic, lactic and succinic acids were also detected. Subsequently *p*-coumarylquinic (2), citramalic, mucic (3) and 2-methyl-2:3-dihydroxybutyric (4) acids have been identified. The present work describes a method used for the quantitative determination of the major and some of the minor acids in juices of fruit at different stages of growth and after varying periods of storage.

Experimental.

Materials.

Changes in the organic acid composition were followed in juices obtained by means of a Kenwood juice extractor from Dabinett fruits picked at three stages before normal maturity. Changes during storage of the fruit were followed in the varieties Dabinett, Sweet Alford and Kingston Black. Sound apples were milled and pressed by the normal cider-making method immediately after picking (Stage I), at the normal stage for cider-making (II) and after several weeks storage but before rotting commenced (III). The Kingston Black fruit was at Stage II immediately after picking, hence Stage I was omitted. Juices from the whole fruit, pulp and peel of the variety Dabinett were examined separately.

Methods.

Total and titratable acidities were determined as previously described (1). The determination of the individual acids was based on the silica gel technique used for other fruit juices (5). The silica gel was prepared as described by Bradfield & Penney (6) and Bradfield, Penney & Wright (7) with certain modifications: an exact equivalent instead of 95% of the equivalent of hydrochloric acid was added to the sodium silicate initially, the gel was not given an intermediate drying, and after the final washing and drying was sieved through a stainless steel 100-mesh B.S.S. sieve.

To obtain good separation of the acids on elution from the silica gel column, pectin was precipitated from the juice samples by addition of 1.5 volumes of acetone to 1.0 volume of juice which had been passed through a column of Amberlite IR 120 (H form) resin. After filtration through a Whatman No. 541 filter-paper, the acetone was removed by distillation under reduced pressure and the juice was concentrated to one-third of its original volume at a temperature not exceeding 40°C.

To determine minor acids on the 3.0 gm. silica gel column it was necessary to concentrate the juice acids still further by the following method. 3.75 ml. of the concentrate were thoroughly mixed with sufficient silica gel to make a dry powder. This was slurried with a 60% tert. butanol-chloroform mixture, equilibrated with water, on to a 6.0 g. silica gel column previously made up by thoroughly mixing the gel with water and slurrying with the same solvent mixture. The acids were eluted from the column with 60% tert. butanol-chloroform, equilibrated with 0.5 N sulphuric acid, until the transparent band reached the foot of the column. One-ninth of the eluate was titrated and the remainder neutralized with sodium hydroxide solution. The neutral solution was concentrated under reduced pressure at a temperature not exceeding 40°C and then taken to dryness in a 10 ml. beaker in a vacuum desiccator.

This mixture of the sodium salts of the acids (from 10 ml. of original juice) was mixed with 0.5 ml. of 0.5 N sulphuric acid and 0.5 g. of silica gel and added to a 2.5 g. silica gel column as previously described (5). The prescribed procedure was carried out with one modification: the fractions of the malic acid peak and of the citramalic acid peak (from the peel extract only) were titrated with 0.05 N instead of with the 0.005 N sodium hydroxide solution used for the other fractions. The peak fractions additional to those reported (5) were chlorogenic acid (fraction no. 23) and citramalic acid (fraction no. 29).

To check the identity of each acid peak, fractions were bulked, concentrated, treated with Amberlite IR 120 (H form) resin and run on paper chromatograms in three solvents (5).

The values obtained for chlorogenic acid were low owing to adsorption of a portion of this acid on the cation-exchange resin used for preliminary treatment of the juice; more accurate values may be obtained by a concentration treatment not involving a cation-exchange resin. (8) Sharp peaks were not obtained for shikimic acid in Dabinett and Kingston Black juices owing to the presence of other acids of similar and lower R_F value in the shikimic acid-containing and adjacent fractions.

Phosphate in the quinic + phosphate peak fractions was incompletely titrated using phenol red as indicator. A correction may be made by adding 60% to the value obtained for phosphate. Quinic acid was determined in a separate aliquot of juice by fractional elution from an IRA 400 (acetate form) column with 0.5 N acetic acid. The quinic acid-containing fractions were taken to dryness *in vacuo* over phosphoric oxide and sodium hydroxide, and titrated.

Results and Discussion.

Changes during growth of Dabinett fruit.

The acid components of the juices at three stages of growth are shown in Table I. A considerable fall in total acidity from Stage A to Stage C is due mainly to decreases in quinic acid + phosphate, chlorogenic acid and, to a smaller extent, in malic acid. Citric acid shows some increase and then decreases to the initial concentration; citramalic acid was not detected until Stage C. Hulme and Woollorton (9), who studied Bramley's Seedling, observed in both citric and malic acid an increase followed by a decrease; as in Dabinett, quinic acid showed a large decrease.

TABLE I.

CHANGES IN ACID CONSTITUENTS OF JUICE DURING GROWTH OF DABINETT FRUIT.
(Milli-equivalents per 100 ml. of juice).

Stage	A	B	C
Date picked	20.7.56	9.8.56	13.9.56
Mean weight of fruit (g.)	7.2	20.7	34.3
Total acidity	8.88	7.13	5.79
Malic acid	3.47	3.26	3.17
Citramalic acid	—	—	0.04
Chlorogenic acid	0.83	0.23	0.19
Citric acid	0.05	0.07	0.05
Quinic acid + H_3PO_4	3.95	2.38	1.26

Changes during storage of the fruit.

The yields and main properties of the juices are given in Table II and the acid composition in Table III. The variety Kingston Black shows a large decrease in acidity due to a fall in malic acid concentration from Stage II to Stage III. The figures for Dabinett (corrected for loss in weight of fruit) show a fall in malic acid concentration and in total acidity, whereas in Sweet Alford there is little change in acidity or in malic acid concentration from Stage I to Stage III. Previous work by Turner (10) on Granny Smith apples stored at 0°C. showed a steady decrease in both titratable acidity and malic acid content. Peel and pulp of Dabinett fruit show a steady decrease in malic acid; Hulme and Wooltorton (11) found a similar result in Bramley's Seedling peel and pulp over the same storage period.

Quinic acid concentration increases by about 50% in the pulp and peel of Dabinett; Bramley's Seedling (11) showed similar results over the same storage period. Shikimic acid concentration in Sweet Alford, as in Bramley's Seedling (11), increases during storage. Chlorogenic acid increases in concentration in Kingston Black and Sweet Alford but remains relatively constant in Dabinett.

Citric acid concentration increases in the peel and pulp of Dabinett, but shows little change in Sweet Alford and Kingston Black. It has been found to rise over the whole period of storage in Granny Smith (10) and Bramley's Seedling (11).

The changes in citramalic acid are the most striking: Dabinett, Sweet Alford and Kingston Black all show increases in the concentration of this acid during storage. In Dabinett most of the citramalic acid is present in the peel, and at Stage III the concentration of this acid in the peel juice is higher than that of malic acid. In the variety Bramley's Seedling (11) the whole of the citramalic acid was present in the peel, and a steady increase in concentration occurred during storage. Dabinett pulp, however, contains a small proportion of citramalic acid (3.5% of that in the peel at Stage I and 9.5% at Stage III).

TABLE II.
CHANGES IN FRUITS AND JUICES DURING STORAGE OF THE FRUITS.

Variety	Sweet Alford			Dabinett			Kingston Black	
	I	II	III	I	II	III	II	III
Maturity stage								
Date of milling and pressing	12.11.56	29.11.56	2.1.57	13.11.57	5.12.56	6.2.57	23.11.56	30.1.57
Loss of weight on storage (as % original weight)	—	2.9	5.2	—	2.7	10.0	—	18.7
Yield of Juice :								
Gallons per ton pressed	165	158	154	163	165	151	150	128
Gallons per ton of original weight	165	154	146	163	160	136	150	104
Specific Gravity of juice	1.048	1.049	1.049 (1.046)	1.052	1.052	1.053 (1.047)	1.065	1.073 (1.057)
pH of juice	4.27	4.21	4.20	4.22	—	4.48	3.45	3.78
% Tannin in juice	0.10	0.10	0.13 (0.12)	0.23	0.24	0.25 (0.22)	0.17	0.19 (0.15)

(Values in brackets corrected for loss in weight of fruit)

TABLE III.
CHANGES IN JUICE ORGANIC ACIDS DURING STORAGE OF THE FRUIT.
(Milli-equivalents per 100 ml. of juice).

Variety	Dabinett									Sweet Alford			Kingston Black	
	I			II			III			I	II	III	II	III
Maturity stage	Whole fruit	Peel	Pulp	Whole fruit	Peel	Pulp	Whole fruit	Peel	Pulp	Whole fruit	Whole fruit	Whole fruit	Whole fruit	Whole fruit
Source of juice	2.55	1.95	1.97	2.55	—	—	2.25	—	—	1.80	1.95	1.95	9.3	6.9
Titratable Acidity	6.12	5.77	5.88	6.12	5.47	5.85	6.42 (5.64)	6.26	6.56	4.03	4.33	4.18 (4.01)	13.6	11.85 (9.2)
Total Acidity	4.05	2.40	4.51	4.08	1.73	4.30	3.67 (3.24)	1.59	3.98	2.73	2.87	2.65 (2.55)	11.3	9.13 (7.1)
Malic acid	0.16	1.10	0.04	0.33	1.88	0.11	0.51 (0.45)	2.31	0.22	0.09	0.11	0.12	0.20	0.45 (0.35)
Citramalic acid	trace	trace	trace	0.10	0.10	0.08	0.11 (0.10)	0.10	0.10	0.04	0.03	0.08	0.09	0.20 (0.16)
Chlorogenic acid	0.04	0.03	0.04	0.06	0.05	0.05	0.11 (0.10)	0.06	0.09	0.04	0.03	0.03	0.12	0.16 (0.13)
Citric acid										0.05	0.05	0.07		
Shikimic acid	0.94	1.21	0.79	0.95	1.16	0.78	1.00	1.35	0.98	0.45	0.56	0.45	0.73	0.87 (0.70)
Quinic acid + H_3PO_4	0.42	0.40	0.44	0.53	0.50	0.48	0.67 (0.59)	0.68	0.70					
Quinic acid	0.52	0.81	0.35	0.42	0.66	0.30	0.33	0.67	0.28					
H_3PO_4														

(Values in brackets corrected for loss in weight of fruit)

There is some indication that inorganic phosphate concentration in both peel and pulp of Dabinett fruit decreases from Stage I to Stage III.

The main changes in acid composition during fruit storage are a fall in total acidity, the proportion of malic acid decreasing while that of the other acids, notably citramalic, quinic and chlorogenic acids, increases.

Storage of the fruit after the normal time of pressing decreases the yield of juice; this loss is due partly to the more pectinous nature of the milled fruit, particularly in Kingston Black. Loss in acidity during storage is proportionately smaller than loss in juice yield.

Summary.

- (1) A method for the quantitative determination of the major and some of the minor non-volatile organic acids in apple-juices, using a silica-gel column, is described.
- (2) During growth of the fruit there was a considerable fall in total acidity due to decreases in the concentrations of quinic and chlorogenic acids and, to a less extent, of malic acid.
- (3) During storage of the fruit, total acidity and the content of malic acid decreased while the contents of citramalic, quinic, shikimic and chlorogenic acids increased: citric acid showed some increase in the variety Dabinett only.

Acknowledgments.

The authors wish to thank Mr. J. H. Llewellyn for valuable assistance.

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A COMPARISON OF SOME VARIETIES OF VEGETABLES PRESERVED BY CANNING AND BY FREEZING : PROGRESS REPORT VI.

By ALICE CRANG, EDNA FILER and MARGARET LEACH.

The quality assessment of varieties of beans, carrots, peas and spinach that have been tested for three or more years is reported. Average markings for colour, texture, flavour and percentage waste in preparation are given. No report was made in 1957 because of the breakdown of the freezer in which the vegetables were held during storage.

Experimental.

Vegetables used. All the vegetables were grown at Long Ashton, picked when at their prime and preserved without delay.

Preservation methods. The methods of canning and freezing beans, peas and spinach were the same as those used previously (1). The carrots were washed to remove any soil, weighed, the leaves removed and the roots scalded for 10 min. in boiling water. Those for canning were partially cooled in water, the skins rubbed off, re-weighed, and 12 oz. packed into a 1 lb. SR can with 5 fl. oz. boiling 2% brine; after exhausting and sealing, the cans were given a 30 min. process at 10 lb. pressure. The carrots to be frozen were scalded and skinned as above, and cooled in ice-cold water before draining, packing and freezing at temperatures below 0°F.

The vegetables were stored for three to four months before tasting, the cans being held at room temperature during this time.

Judging. The method of scoring has been altered since the last report (1), colour, texture and flavour each being marked out of a maximum of 5 instead of 10 as previously. The comparable markings are as follows :

<i>Present System</i>	<i>Old System</i>	<i>Quality Grading</i>
5	10	Colour, texture or flavour : excellent.
4	9	Colour, texture or flavour : very good.
3	8	Colour, texture or flavour : normally good.
2	7	Colour : some unevenness, or slightly too pale or too dark. Texture : slight unevenness, softness, toughness or breakdown. Flavour : rather weak.
1	6	Colour : too pale, too dark or patchy. Texture : uneven, too soft or too tough but passable. Flavour : very weak or slightly foreign, but not objectionable.
0.1	5	Colour, texture or flavour very poor but just passable.
0	below 5	Unacceptable.

The tasting panel consisted of 12-15 members of staff, most of whom have been judging regularly in these trials for several years.

Results.

Beans, Broad.

The 10 varieties tested during 1958 all gave satisfactory products when frozen : they were not canned, as previous results on canning varieties other than Triple White had been unsatisfactory.

The varieties Aquadulce Claudia, Dwarf Gem (Beck's), Eclipse and Exhibition (Bunyard's), tested for three years, all gave satisfactory frozen products ; the average marking for each variety is given in Table I.

TABLE I.
MEAN MARKS FOR QUALITY OF BEAN VARIETIES TESTED FOR THREE SEASONS.
(Maximum 5).

Variety	Colour		Texture		Flavour		Waste	
	*C.	*F.	*C.	*F.	*C.	*F.	% Total Weight	
							Average	Range
<i>Broad Beans</i>								
Aquadulce Claudia	—	2.9	—	2.8	—	3.2	69	61-75
Dwarf Gem	—	3.3	—	2.5	—	2.9	61	60-62
Eclipse	—	3.2	—	3.0	—	2.9	60	53-68
Exhibition	—	2.9	—	3.3	—	3.1	67	66-70
Mean 10 varieties 1958 ..	—	3.4	—	3.1	—	3.2	67	56-74
" 7 " 1956 ..	—	2.8	—	2.8	—	2.9	62	53-70
" 5 " 1955 ..	—	2.9	—	1.8	—	3.3	64	45-75
<i>French Dwarf</i>								
Golden Wax (stringless) ..	0.6	2.1	2.6	3.1	1.6	2.5	9	8-10
Granda	1.9	2.6	2.2	2.8	1.9	2.8	8	6-10
Perpetual	2.2	2.9	2.4	2.7	2.2	2.6	30	27-31
Top Crop	2.2	2.4	2.9	3.2	1.5	2.4	9	6-12
<i>French Climbing</i>								
Blue Coco	0.9	3.3	2.9	3.3	1.3	2.4	24	22-26
Blue Lake (stringless) ..	2.1	3.1	3.2	3.2	1.7	2.5	9	6-12
Guernsey	1.8	3.1	2.3	3.0	1.7	2.4	23	21-25
July Climbing	2.8	2.9	3.5	3.2	1.9	2.8	8	6-11
Purple Podded	1.3	2.9	2.6	2.8	1.7	2.7	23	22-24
Mean 17 varieties 1958 ..	1.7	3.0	2.7	3.2	2.1	2.8	—	—
Stringless	—	—	—	—	—	—	12	9-14
Others	—	—	—	—	—	—	26	22-31
Mean 15 varieties 1956 ..	1.7	2.7	2.5	3.0	1.7	2.6	—	—
Stringless	—	—	—	—	—	—	8	6-11
Others	—	—	—	—	—	—	28	19-33
Mean 10 varieties 1955 ..	2.0	2.4	2.7	2.7	1.8	2.1	—	—
Stringless	—	—	—	—	—	—	8	6-9
Others	—	—	—	—	—	—	24	21-28

*C = Canned ; F = Frozen.

Beans, French.

Of the 17 varieties tested in 1958, 12 were of dwarf and the remainder of climbing habit: 8 of the dwarf and 2 of the climbing varieties were tested as "stringless," having only the tips removed in preparation, whereas the others needed stringing when mature. Nine of these varieties have been tested for three seasons; the scores for these are given in Table I.

The beans were prepared by stringing if necessary, and in dwarf varieties the beans were cut in half lengthwise. The climbing beans were prepared in two ways—either cut into thin slices or diagonally into slices about 1" thick. There was a slight preference in colour for the thinly sliced beans, but as there was otherwise little difference due to method of cutting, the individual results are based only on the thinner slices.

The frozen beans were once again preferred to the canned ones for colour and flavour.

The varieties Blue Coco, Perpetual and Purple Podded were stringless when young, but when mature these three and Guernsey needed stringing, whereas the other five varieties reported remained stringless when mature.

July Climbing proved the most satisfactory of the varieties for canning, yet its flavour was rather weak. Golden Wax cannot be recommended for preserving, its colour being very poor when canned and not generally liked when frozen, whereas Blue Coco, although poor in colour when canned, was quite satisfactory when frozen. The dwarf beans were rated a little below the climbing French beans for colour when frozen, but variations in texture and flavour between the varieties were small. None of these varieties gave such a bright green colour when frozen as the Runner beans Scarlet Emperor and Kelvedon Wonder reported previously (2).

Carrots.

The five varieties of carrot tested for three seasons (see Table II) were preserved whole as soon as the roots were large enough to pull. Of these, Early Nantes was thought the best when frozen; it also had the best appearance when canned, though its flavour was not rated very highly. The appearance of Perfect Gem was also very good when frozen, and good when canned; Scarlet Intermediate and Scarlet Horn rated next, while Golden Ball was considered of only fairly good colour whether frozen or canned. The differences between the ratings of the canned and frozen samples were less than with most kinds of vegetables; the canned samples of Golden Ball were in fact considered slightly better than the frozen ones.

Although the average waste in preparation was 30%, this was reduced to 8% if the leaves were cut off when the carrots were pulled. Early Nantes and Perfect Gem were the least wasteful and Golden Ball the most wasteful in preparation.

Peas.

The three varieties of peas, Laxton's Superb, Meteor and Rentpayer, reported after three years' testing (Table II), yielded poor products when canned under domestic conditions, but had very good colour and satisfactory flavour when frozen. Among varieties reported previously (1), Onward and Thomas Laxton were rated higher for colour, texture and flavour when frozen,

but the waste on preparation was higher, being 75% and 72% respectively for these varieties against 64% and 65% for Laxton's Superb and Rentpayer, and only 55% for Meteor.

TABLE II.
MEAN MARKS FOR QUALITY OF CARROT, PEA AND SPINACH VARIETIES TESTED
FOR THREE SEASONS.
(Maximum 5).

Variety	Colour		Texture		Flavour		Waste	
	*C.	*F.	*C.	*F.	*C.	*F.	% Total Weight	
							Average	Range
<i>Carrots</i>								
Early Nantes	2.8	4.0	2.7	2.9	1.7	2.8	28	23-33
Golden Ball	2.3	2.1	3.0	2.8	2.2	2.0	39	28-46
Perfect Gem	2.7	3.8	3.1	3.0	1.9	2.3	23	21-26
Scarlet Horn	2.3	3.1	3.0	2.8	1.9	2.0	32	23-36
Scarlet Intermediate	2.7	3.2	2.7	2.7	1.7	2.6	35	—
<i>Peas</i>								
Laxton's Superb	1.4	4.0	1.9	1.9	0.9	2.6	64	—
Meteor	1.5	3.9	2.2	2.5	1.2	2.7	55	51-59
Rentpayer	1.8	3.8	2.2	3.1	1.3	2.7	65	62-70
Mean 8 varieties 1958 ..	1.9	4.1	2.1	3.3	2.2	3.2	60	55-65
Mean 4 „ 1956 ..	1.1	3.6	2.1	2.6	0.6	2.6	64	58-73
Mean 6 „ 1955 ..	1.8	3.9	2.3	2.6	1.5	2.8	62	51-74
<i>Spinach</i>								
Goliath	0.8	3.4	2.9	3.0	2.2	2.5	18	12-21
New Zealand	0.9	3.4	3.0	3.2	1.7	2.5	7	4-9
Zenith XXX	1.1	3.6	3.1	3.0	2.1	2.9	16	12-19
Mean 4 varieties 1958 ..	1.5	3.6	3.1	3.4	2.3	2.8	15	8-20
Mean 5 „ 1956 ..	0.8	3.5	2.8	3.2	1.7	2.9	11	9-12

*C = Canned ; F = Frozen.

Spinach.

Goliath, New Zealand and Zenith XXX, the 3 varieties reported in Table II, gave good frozen products. The colour of the canned spinach was very poor, despite the addition of artificial colour, and canning cannot be recommended, although the texture was good and flavour fairly good. The quality of these varieties is similar to that of Giant Savoy Leaf, Perpetual and Prickly New Giant reported previously (2).

The variety New Zealand had small thick leaves, and the wastage figures were low, but it did not grow well in the wet conditions prevailing in the summer of 1958.

Summary.

This report gives the quality assessment and wastage during preparation of 10 varieties of broad bean preserved by freezing, and of 17 varieties of French bean, 5 of carrots, 8 of peas, and 4 of spinach preserved by canning and by freezing. Comments are made on 24 individual varieties that have been tested for 3 seasons.

Acknowledgments.

We wish to thank Miss P. Davey for help in preserving the vegetables, and the members of the tasting panel who helped with the assessment.

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THE DESSERT AND PRESERVING QUALITIES OF SEVEN GOOSEBERRY VARIETIES.

By ALICE CRANG and BARBARA A. RAKE.

For the past few years tests have been made of the dessert and preserving qualities of a number of gooseberry varieties grown at Long Ashton. The present report is limited to varieties that have been tested during three seasons. As it has been found that the canning quality of gooseberries is similar to their bottling quality (1), remarks on bottled fruit may be assumed to apply also to fruit canned by normal commercial methods.

Experimental.

Fruit was picked green, about ten weeks after fruit set, and bottled in 1-lb. Kilner-type jars. Each jar held 9 oz. of prepared fruit, which was covered with 30° Brix syrup at approximately 140°F. The jars were immersed in warm water (100°F.), heated to 190°F. in 25-30 minutes and held at that temperature for 10 minutes before removing and screwing down. The bottled fruit was stored at 36-40°F. for five months before tasting.

Tests of dessert quality were made on fruits picked ripe and tasted within twenty-four hours of picking. Tasting was carried out by a panel, normally of twelve people, using the scoring schedule for colour, texture and flavour given on page 175.

Results.

Table I gives the marks awarded by the tasting panel for colour, texture and flavour of dessert and bottled fruit, as mean values for three years' assessment. None of the varieties reached an average score for texture and flavour. Apart from Broom Girl (normally unattractive), scoring for colour of fresh fruit was average or slightly above average.

In order to be commercially suitable for picking green, the berries should reach a fair size early in the season; this does not occur with all varieties. Table I shows which varieties have this character. For use in private gardens,

TABLE I.

DESSERT AND PRESERVING QUALITIES OF SEVEN GOOSEBERRY VARIETIES.

Mean marks (maximum 5) for three seasons.

Variety		Colour	Texture	Flavour	*Size	Colour (ripe)	Hairiness
Bedford Red	Bottled	2.6	2.3	2.6	Large	Dark crimson	Very few bristles
	Dessert	3.3	2.7	2.5	Large		
Bellona	Bottled	2.1	2.8	2.7	Large	Medium green, red spots	Glabrous
	Dessert	3.0	2.6	2.1	Medium-large		
Broom Girl	Bottled	2.5	2.1	2.6	Large	Dull yellow	Few bristles
	Dessert	2.0	2.6	2.7	Large		
Cousen's Seedling	Bottled	2.2	2.6	2.9	Small	Golden yellow	Many bristles
	Dessert	3.9	2.8	2.7	Medium		
Early Sulphur	Bottled	3.0	2.5	2.5	Small	Golden yellow	Many bristles
	Dessert	3.2	2.5	2.6	Small-medium		
Golden Drop	Bottled	2.8	2.7	2.6	Small	Golden yellow	Many bristles
	Dessert	3.0	2.7	2.3	Small		
Whinham's Industry	Bottled	2.2	2.5	2.4	Large	Dark crimson	Very few bristles
	Dessert	3.1	2.6	2.6	Large		

*Size bottled : Small < 4 g. ; Medium=4.5 g. ; Large > 5 g.

Size dessert : Small < 8 g. ; Medium=8-13 g. ; Large > 13 g.

where economics are not so important, a small-sized berry might be used for picking green provided its flavour were good enough. It is doubtful from the results whether Cousen's Seedling, Early Sulphur and Golden Drop are worthwhile varieties to bottle under any conditions.

Table I also shows colour, hairiness of skin and size of ripe berries of the varieties tested ; these may have an influence on preference. Red and yellow berries usually appear riper and more appetising than green or white ones ; some yellows (e.g. Broom Girl) are dull and muddy-looking and very unattractive. Hairiness of the berry may detract from its dessert quality, but hairs do not appear to have much influence on preference when the berries are cooked.

Acknowledgments.

Thanks are due to members of Station staff for help in preserving and judging the fruit.

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A NOTE ON COVERS FOR PICKLES AND JAMS.

By ALICE CRANG and MARGARET LEACH.

Since 1941, when a series of tests was made on methods of covering jam to reduce mould growth (1), several new covers have come on the market. The efficiency of some of those available for domestic use on pickle and jam jars has been tested and the newer jam covers have been compared with those recommended previously. Three of the pickle covers require special jars, but the other five are suitable for use on standard jam jars.

Experimental.

Pickles.

Each type of pickle cover was tested by filling five jars with a weighed amount of vinegar of 4.3% acetic acid content, covering at once, and holding for 7 weeks at 25°C. before re-weighing and determining the acid content of each jar. A similar test was made with three jars of chutney made from a standard recipe and stored for 10 weeks at 25°C. The pickle covers used were :

- (a) "Alkathene" : semi-rigid covers with a shallow rim made to fit standard jam jars ;
- (b) Metal with ceresin-covered discs : 4-lug lacquered metal with an inner disc of waxed cardboard and a ceresin vinegar-resistant layer, made for use with straight-sided glass pickle jars ;
- (c) Metal plastic-lined screw-cap : screw-cap with a metal and plastic inner disc, used extensively on commercial pickle jars ;
- (d) Plastic screw-cap : rigid plastic, with an inner plastic-coated cardboard disc, for use on screw-cap jars ;
- (e) Polythene-coated vegetable parchment : polythene layer 0.001" thickness on vegetable parchment (57 g. per sq. m.). The material was damped on the parchment surface and tied tightly on jam jars with this layer uppermost ;
- (f) As (e), with the polythene layer damped and placed uppermost ;
- (g) Vegetable parchment over ceresin and waxed cardboard discs : the two discs were fitted over the mouth of standard jam jars with the ceresin next to the vinegar, and vegetable parchment was tied on tightly, after damping the upper surface ;
- (h) Vegetable parchment : tied on tightly after damping the upper surface.

Greaseproof paper alone, greaseproof paper with a covering of strong cotton material dipped in melted paraffin wax, and vegetable parchment with a ceresin disc but no cardboard disc, were compared with the covers (a) to (h) in preliminary trials.

Jams.

The vegetable parchment and cellulose tissues, previously found to be the most satisfactory of the covers tested (1), have been compared with four newer covers. Nine kinds of jam with 60% added sugar content were used. The jars were filled and waxed tissues pressed evenly over the surface of the jam with the minimum of delay. In one series the covers were put on at once, and in another after covering with a cloth and leaving the jam to cool overnight.

In order to test the covers under bad storage conditions, the jars were exposed to mould spores before covering, and some of the jams were stored for 10 weeks in warm, damp, mould-impregnated boxes, while the others were stored for 32 weeks in a cool damp store in which mould was prevalent. All the jars were re-weighed and examined for spoilage after storage.

The covers used were :

- (a) "Alkathene" (*see* Pickle Covers (a) ;
- (b) Cellulose tissues : Cellophane or Diophane covers were damped on the upper surface and tied on tightly ;
- (c) Compressed paper caps : two makes were tested ; both were pressed over the mouths of the jars ;
- (d) Polythene : medium weight polythene was tied on tightly ;
- (e) "Regor" glass caps : glass lids, sold for use with jam jars, with rubber bands and spring clips ;
- (f) Vegetable parchment (*see* Pickle Covers (h)).

Results.

Pickles.

The losses of moisture from the jars containing vinegar and chutney, using covers (a) to (g), are given in Table I. It will be seen that the commercial metal screw-cap (c) was the most satisfactory in preventing moisture loss, but that losses were small with the other metal cap (b) and with the two plastic caps (a) and (d). The losses were greater, but not serious, with the polythene-parchment covers when the parchment was uppermost (e) and in the parchment with a ceresin and cardboard disc (g), but they were considerably greater when polythene-parchment was used with the polythene on top (f). Very serious losses of moisture occurred from both the vinegar and chutney stored with vegetable parchment covers alone (h) ; the losses in weight from the five jars of vinegar were 60.5, 66.9, 91.0, 92.0 and 94.7%, whilst those from the chutney ranged from 31.7 to 41.4%.

In preliminary tests, greaseproof paper had proved even less effective than vegetable parchment and, even when a layer of cotton dipped in melted paraffin wax was put over the greaseproof paper, losses were greater than with covers (a) to (d). The omission of the cardboard disc from treatment (g) was found to cause increased evaporation.

Covers (a) to (d) inclusive were most effective in preventing loss of acetic acid during the period of the test though, on longer storage, the vinegar was found to attack the metal of the covers (b). About half the acetic acid was lost through the polythene-coated parchment when the parchment was on top, but considerably more with the polythene on top, the losses from vegetable parchment covering ceresin and cardboard discs falling between these two amounts.

Unexpected results were obtained from the vegetable parchment used alone (h). Despite exceptionally heavy losses of moisture through evaporation, there was a comparatively small loss of acetic acid, with the result that the concentration of acetic acid in the remaining solutions had increased from 4.3% to 10.3, 12.1, 40.6, 48.0 and 71% respectively, with losses of total acetic acid from the original contents varying from 0.2 to 0.6%. It seems, therefore, that vegetable parchment, when tied on tightly alone, acts as a semi-permeable membrane, allowing the passage of moisture but retaining acetic acid.

TABLE I.

LOSS OF WEIGHT AND ACETIC ACID AFTER STORAGE AT 25°C.

Vinegar stored 7 weeks : Mean of 5

Chutney „ 10 „ „ „ 3

Cover		Loss of weight %		Residual acetic acid %		Total loss of acetic acid %
		Mean	Range	Mean	Range	Mean
a. "Alkathene"	Vinegar	1.1	0.7-1.5	4.3	4.3-4.3	nil
	Chutney	1.0	0.6-1.4			
b. Metal with ceresin discs	Vinegar	1.1	0.8-1.5	4.3	4.3-4.3	nil
	Chutney	0.4	0.2-0.5			
c. Metal plastic-lined screw-cap	Vinegar	0.3	0.0-0.7	4.2	3.9*-4.3	0.1*
	Chutney	0.2	0.2-0.3			
d. Plastic screw-cap	Vinegar	0.9	0.7-1.0	4.3	4.3-4.3	nil
	Chutney	0.9	0.7-1.2			
e. Polythene-coated vegetable parchment ; parchment on top	Vinegar	4.0	2.2-5.7	2.2	0.7-3.4	2.1
	Chutney	1.7	1.1-2.3			
f. Polythene-coated vegetable parchment ; polythene on top	Vinegar	15.1	8.5-21.6	0.8	0.4-1.0	3.6
	Chutney	2.6	2.5-2.7			
g. Vegetable parchment over ceresin and cardboard discs	Vinegar	4.7	3.3-7.7	1.8	1.2-2.2	2.6
	Chutney	2.0	1.5-2.5			

*Loss from 1 jar only. For Cover (h) see text above.

Jams.

Under the damp storage conditions of the test there was no significant loss of moisture through any of the covers tested ; in fact a few jars gained weight during storage.

The amount of mould growth is given in Table II. In no instance had the mould penetrated the tissue on the jam.

When the jams were covered hot, it will be seen that under the stringent test conditions there was no mould on the inside with the Regor covers and slight mould on only one jar covered with Alkathene, cellulose tissue or vegetable parchment, but on more of the jars covered with compressed paper or polythene. The jams covered cold showed a higher proportion of mould on the inside, but the vegetable parchment (one heavy mould) and cellulose tissue (one heavy and one slight mould) again proved the best of the series. A number of jars with each kind of cover had mould on the outside.

Additional tests were carried out on underfilled jars, three covers of each kind being put on hot and another three on cold jam. With cellulose tissue there was no mould inside whether the jams were covered hot or cold ; with compressed paper there was none when covered hot, but with all other treatments mould growth was present in some of the jars. Taking all the covers into consideration, the percentage of jars showing mould growth was :

TABLE II.
MOULD GROWTH ON JAMS WITH DIFFERENT COVERS.
(Nine jars of each treatment; well filled).

Cover		Mould growth		
		Heavy	Slight	None
" Alkathene "	Covered hot	0	1	8
	Covered cold	1	2	6
Cellulose tissue	Covered hot	0	1	8
	Covered cold	1	1	7
Compressed paper	Covered hot	1	2	6
	Covered cold	2	2	5
Polythene	Covered hot	3	0	6
	Covered cold	2	0	7
" Regor "	Covered hot	0	0	9
	Covered cold	1	2	6
Vegetable parchment	Covered hot	0	1	8
	Covered cold	1	0	8

		Mould growth		
		Heavy	Slight	None
Filled jars, covered hot		6%	10%	84%
" " " cold		15%	13%	72%
Underfilled jars, " hot		17%	17%	66%
" " " cold		17%	33%	50%

Although the best results were obtained from the jars covered hot, it is realized that it is often difficult to handle hot jam and to cover it quickly enough when a large quantity is being made. Under these conditions it is better to cover the jam when cold and to use materials, such as cellulose tissue or vegetable parchment, that allow the evaporation of a certain amount of moisture.

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Month	AIR TEMPERATURE (F°.)						RAINFALL (")			SUNSHINE (hours)		SOIL TEMPERATURE at 09-00 G.M.T.		
	Means		Diff. from normal	Max.	Min.	No. of ground frosts	Total	Per cent. of average	Most in a day	Daily mean	Per cent. of average	4"	8"	24"
	A	B												
January	45.7	33.6	-0.8	54	13	15	2.78	83	0.66	1.84	117	39.0	40.1	42.9
February	48.8	37.5	+2.3	58	29	13	4.38	170	1.26	1.75	72	41.4	42.3	44.0
March	46.8	33.8	-3.6	60	20	17	1.21	53	0.47	2.46	89	38.5	39.9	42.6
April	53.7	38.0	-1.8	71	26	13	1.25	57	0.51	4.72	86	45.0	44.5	46.5
May	60.5	45.7	0.0	75	41	1	3.58	139	0.76	6.33	99	54.1	53.0	54.7
June	65.6	50.2	-0.6	74	41	0	3.63	164	2.16	5.64	81	60.0	60.6	60.1
July	69.0	53.4	-0.3	78	42	0	1.99	64	0.40	5.63	88	63.5	62.5	63.2
August	67.7	54.5	-1.1	76	46	0	3.07	83	0.33	4.12	69	62.6	62.1	63.0
September	66.3	52.7	+2.2	77	42	0	5.47	168	1.33	3.80	81	59.1	59.3	58.8
October	58.7	46.5	+1.5	62	35	4	3.18	85	1.07	3.24	99	51.8	52.9	56.6
November	49.7	39.1	-0.4	60	28	11	2.42	66	0.49	1.38	73	44.4	45.8	47.8
December	46.4	37.3	+0.6	55	29	16	3.66	103	0.57	0.82	56	40.7	41.5	44.5
Totals or Means	56.6	43.5	-0.1			90	36.62	103		3.56	84	50.0	50.4	52.0

G. E. CLOTHIER.

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THE JOURNAL OF HORTICULTURAL SCIENCE

The Journal of Horticultural Science, which is sponsored by the Long Ashton Research Station and the East Malling Research Station, welcomes contributions on horticultural science from Research Institutes at home and abroad. Papers for publication and all communications should be sent to the Editor at East Malling Research Station, Maidstone, Kent.

Papers in the current Volume 34 (1959) come from both the sponsor Stations, from Bayfordbury, Wellesbourne, Rustington and Mylnefield, from Reading and Nottingham Universities and from the N.A.A.S. There are also papers from the Johnstown College, Eire, the Citrus Station, Nelspruit, S. Africa and the Cocoa Research Institute, Ghana.

In this Volume will be found two papers dealing with wood infections in apple due to *Gloeosporium perennans*, a further paper on fruit development in relation to plant hormones, in this instance dealing with figs, and one on *M. platycarpa* as an indicator of a virus of apples. Other subjects pertaining to fruit research are apple rootstocks and their resistance to *Phytophthora cactorum*; root development of apples and the effect of magnesium deficiency on their cropping, the effects of environment on raspberries and of biennial bearing on the chemical composition of apple leaves; the performance of selected navel oranges; temperature as affecting the frost resistance of apple flowers, and also chrysanthemums; control of strawberry eelworms, star crack virus of apples; effects of shade reduction and manuring on cocoa and a study of the design of experiments on that variable crop. Vegetable papers deal with the development of tomatoes, weed effects on onions and beet, and irrigation on cauliflowers; an instrument for measuring the firmness of brussels sprouts is described.

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